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A new cryptic species of the *Pristimantis lacrimosus* group (Anura, Strabomantidae) from the eastern slopes of the Ecuadorian Andes

Julio C. Carrión-Olmedo[1], Santiago R. Ron[1]

1 *Museo de Zoología, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre y Roca, Aptdo. 17-01-2184, Quito, Ecuador*

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Corresponding author: Santiago R. Ron (santiago.r.ron@gmail.com)

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Abstract

With 566 species, the neotropical genus *Pristimantis* is the most speciose vertebrate genus. As a result of its striking diversity, taxonomic reviews remain a challenge. Herein, we present an updated phylogeny of the *Pristimantis lacrimosus* group and describe a new species from Llanganates and Sangay National Parks. We also report, for the first time, the phylogenetic position of *Pristimantis degener*, *P. eugeniae*, *P. katoptroides*, and *P. petersi*. Based on our phylogeny, we add two species to the *Pristimantis lacrimosus* group. Through the integration of molecular and bioacoustic evidence, we describe a new species which was hidden under "*Pristimantis petersi*". *Pristimantis petersioides* **sp. nov.** is most closely related to *Pristimantis petersi* and an undescribed species from Peru. It can be distinguished from *P. petersi* by its advertisement call and large genetic differences (uncorrected *p*-genetic distances 7.9% to 8.4% for gene 16S). Moreover, the new species and *P. petersi* are not sister species. We suggest assigning the new species to the Endangered Red List category because it has a small distribution range with deforestation as result of agriculture and other anthropogenic influences.

Key Words

Amphibia, Bioacoustics, Conservation, Diversity, National Parks, Phylogeny, Taxonomy

Introduction

The genus *Pristimantis* Jiménez de la Espada, 1870 has bewildered scientists for its striking diversity. Comprising 566 Neotropical species it is the most speciose vertebrate genus (Hedges et al. 2008; Frost 2021). In Ecuador, this genus encompasses more than one third of all anuran species, with 233 out of 640 species (Ron et al. 2019).

The astounding species richness of *Pristimantis* has been attributed to terrestrial breeding – direct embryonic development without a tadpole stage (Padial et al. 2014) – and the appearance of geographic barriers as result of the Andean uplift (Lynch and Duellman 1997; Mendoza et al. 2015). However, other sympatric Andean genera with similar reproductive mode (e.g., *Strabomantis* Peters, 1863) and age are not as diverse suggesting that terrestrial breeding and Andean uplift are not the only factors explaining the high diversity of *Pristimantis*.

The number of described species of *Pristimantis* is increasing rapidly as result of the use of DNA sequences allowing the discovery of a large number of cryptic species. DNA sequences helps to achieve better informed taxonomic decisions and speed up species discovery with more than 40 species of *Pristimantis* (e.g. Ortega et al. 2015; Guayasamin et al. 2017; Páez and Ron 2019) described in Ecuador in the last five years. In some clades, the number of undescribed species outnumbers described species (e.g., Ortega et al. 2015; Páez and Ron 2019) suggesting that there could be hundreds of undescribed species of *Pristimantis*.

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Taxonomy within this group have been problematic and unstable because most species descriptions have been based on morphological traits (Lynch and Duellman 1997), which can be highly variable and homoplastic (e.g. Guayasamin et al. 2015; Páez and Ron 2019). Taxonomic problems in *Pristimantis* are still pervasive and are still far from being fully resolved (e.g., Páez and Ron 2019; Reyes-Puig et al. 2019; Moravec et al. 2020).

One clade of *Pristimantis* containing undescribed species is the *Pristimantis lacrimosus* species group (Ron et al. 2020). Hedges et al. (2008) proposed it as monophyletic based on molecular data from only three species. However, more recent phylogenies with better species sampling showed that this group was paraphyletic (Padial et al. 2014; Rivera-Correa and Daza 2016). Rivera-Correa and Daza (2016) identified two non-sister clades within the *Pristimantis lacrimosus* species group, "clade A" endemic to Colombia and "clade B" composed by species from Central America, Ecuador, and Peru. González-Durán et al. (2017) proposed the *Pristimantis boulengeri* group for "clade A" and hypothesized that "clade B" corresponds to the *P. lacrimosus* species group. That assignment was questioned by Rivera-Correa and Daza (2020) but was demonstrated as correct by Ron et al. (2020) who included in their phylogeny, for the first time, the type species for the group, *P. lacrimosus* (Jiménez de la Espada, 1875). Ron et al. (2020) also described three new species and redefined the group to include all descendant species of the most recent common ancestor of *P. eremitus* (Lynch, 1980) and *P. lacrimosus* for a total of 36 formally described species.

Despite these advances, there still are species of the *Pristimantis lacrimosus* group which have never been included in molecular phylogenies. One of them is *Pristimantis petersi* (Lynch & Duellman, 1980). Since its description, this species suffered several taxonomic changes. Lynch (1991) changed its name to *Eleutherodactylus petersorum* to avoid homonymy with a Mexican species with the same epithet, which now is known as *Eleutherodactylus nitidus* (Peters 1870). Additionally, Lynch (1996) created the name *Eleutherodactylus johnwrighti,* as an amend to the previous epithet *petersorum*. Afterwards, Frost (2009) applied Article 59.4 of the International Code of Zoological Nomenclature making *Eleutherodactylus johnwrighti* and *Eleutherodactylus petersorum* invalid replacement names because the taxa in question are not congeneric and recovered the first epithet used for this species.

Pristimantis petersi has been usually assigned to the *P. lacrimosus* species group. Lynch and Duellman (1980) placed it in the P. unistrigatus group, *P. lacrimosus* assembly. Later, Lynch and Duellman (1997) placed it in the *unistrigatus* group, *martinicensis* series. Hedges et al. (2008) proposed this species as a member of the *Pristimantis lacrimosus* group and Padial et al. (2014) and Rivera-Correa and Daza (2016) followed this proposal but without including *Pristimantis petersi* in their phylogenies.

Pristimantis petersi holotype (KU 143508) is from 16.5 km NNE of Santa Rosa, Napo Province, 1700 m. However, most specimens used in the species description by Lynch and Duellman (1980) were from other populations including localities in the central Andes of Colombia (Huila and Putumayo Departments), and central Ecuador (Napo and Pastaza provinces). As currently defined, *Pristimantis petersi* is considered to have a wide distribution from the central Andes of Colombia (Lynch and Duellman 1980; Mueses-Cisneros 2005; Stuart et al. 2008) to the eastern slopes of the Ecuadorian Andes, from Sucumbíos to Morona Santiago Provinces at altitudes ranging between 1400–2000 m a.s.l (Brito et al. 2017; Ron et al. 2019). Lynch and Duellman (1980) mentioned that *Pristimantis petersi* exhibits body size variation throughout its distribution range and, remarkably, realized that individuals from the upper Pastaza trench were larger than individuals from the type locality, Ecuador, and suspected that populations from Pastaza may represent another species.

The wide geographic distribution of *Pristimantis petersi* suggest that it may be a species complex. Recent reviews of Andean *Pristimantis* suggest that most species have highly restricted distributions. For example, in the subgenus *Huicundomantis* Páez & Ron, 2019 all species had distribution ranges below 5000 km^2 and the seemingly large distribution of "*P. phoxocephalus*" and "*P. riveti*" were an artifact of the combined distribution of several cryptic species (Páez and Ron 2019). Similarly, "*P. calcarulatus*" was considered to be distributed in the Andes from central Ecuador to southern Colombia (Hutter and Guayasamin 2015). However, genetic and morphological evidence demonstrated that "*P. calcarulatus*" was a complex of three different species, each with a small distribution range. Similar results have been found within "*P. orestes*", "*P. ornatissimus*" (Guayasamin et al. 2017; Urgiles et al. 2019), and *P. ventrimarmoratus* (Moravec et al. 2020). It is unclear if species of Andean *Pristimantis* with large distributions (> 10000 km^2) actually exist. We are unaware of any species of *Pristimantis* with such distribution withstanding a taxonomic review based on genetic and phenotypic characters. The available evidence suggests that species with seemingly large distributions, like *P. petersi*, may represent species complexes.

The existence of cryptic diversity within *P. petersi* is also suggested by reports of body size differences among populations of *P. petersi* (Lynch and Duellman 1980; Brito et al. 2017). Recent fieldwork conducted by field staff of the QCAZmuseum from Pontificia Universidad Católica del Ecuador resulted in collections of *P. petersi* near its type locality and in the discovery of populations of a species similar to *P. petersi* in Sangay National Park and Llanganates National Park. Through the integration of genetic and bioacoustic data with an exhaustive population sampling, we demonstrate that those populations are distinct from *P. petersi*. We describe the new species and review the content and phylogenetic relationships of the *Pristimantis lacrimosus* group.

Materials and methods

Nomenclature

Taxon names follow Duellman and Lehr (2009) and AmphibiaWeb (2019).

DNA extraction, amplification and sequencing

We inferred the phylogenetic relationships of the new species and closely related taxa based on DNA sequences of one nuclear gene: Recombination activating gene 1 (RAG-1) and three mitochondrial genes: 12S rRNA (12S), 16S rRNA (16S), NADH dehydrogenase subunit 1 (ND1) and their flanking tRNAs. DNA was extracted from muscle or liver tissue preserved in 95% ethanol using standard Guanidine thiocyanate extraction protocols. We used polymerase chain reaction (PCR) to amplify DNA fragments. Primers used for amplification of 12S were t-Phe-frog and t-Val-frog (Wiens et al. 2005), 12SZ-L and 12SK-H (Goebel et al. 1999), for 16S, primers were 12sL13 (Feller and Hedges 1998), 16L19 and 16H36E (Heinicke et al. 2007), for ND1, primers were WL379, WL384, t-Met-frog and 16S-frog (Moen and Wiens 2009), for RAG1, primers were R182, R270, Rag1FF2, Rag1FR2 (Heinicke et al. 2007). PCR amplification was performed under standard protocols and sequenced in both directions by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea). All sequences were assembled in Geneious 7.1.7. and then exported to Mesquite version 3.40 where each genomic region was aligned separately using default parameters in Muscle (Edgar 2004). Unambiguous alignment errors were corrected manually in Mesquite (Maddison and Maddison 2018). The aligned matrix is available in https://doi.org/10.5281/zenodo.3785738. To calculate the uncorrected pairwise genetic *p*-distances of 16S we used MEGA7 on a fragment of 653 pb (Kumar et al. 2016).

We included 156 GenBank sequences of congeneric species. To find relevant sequences in GenBank, we made a 16S BLASTn search with the sequences of the new species (Table 1). These searches showed that the most similar sequences belong to species from the *P. lacrimosus* group: *P. schultei* (Duellman, 1990) (identity 88.62%, accession EF493681), *P. bromeliaceus* (Lynch, 1979) (identity 86.92%, accession EF493351.1) and others such as *P. galdi* Jiménez de la Espada, 1870 (identity 87.76%, accession EU186670.1), and *P.* cf. *mendax* (Duellman, 1978) (identity 87.07%, accession EU186659.1). Therefore, we included sequences used in previous studies on *P. lacrimosus* group (e.g. Arteaga et al. 2013; Padial et al. 2014; Ortega-Andrade et al. 2015; Rivera-Correa and Daza 2016; Chávez and Catenazzi 2016; Shepack et al. 2016; Guayasamin et al. 2017; Ron et al. 2020; Rivera-Correa and Daza 2020). Samples of *Niceforonia nigrovittata* (Andersson, 1945), *N. elassodisca* (Lynch, 1973), and *P. w-nigrum* (Boettger, 1892) were set as outgroups. The combined DNA matrix had up to 4067 bp and 140 terminals.

Phylogeny

The phylogeny was inferred using Maximum Likelihood as optimality criterion. To choose the substitution models that best adjusted to our sequences, we used Model Finder under the command MFP+MERGE (Kalyaanamoorthy et al. 2017; Chernomor et al. 2016) as implemented in IQ-TREE 1.6.8 (Nguyen et al. 2015). We partitioned the sequences by gene and by codon position in coding genes. For the ML search we used IQ-TREE 1.6.8 (Nguyen et al. 2015) under default values. To assess branch support we obtained ultrafast bootstrap values from 2000 pseudoreplicates and 10000 iterations as maximum number to stop (commands -bb 2000 and -nm 10000 in IQ-TREE) and SH-like approximate likelihood ratio test (SH-aLRT) with 1000 replicates (-alrt 1000 command, Guindon et al. 2010). We considered that branches with bootstrap values > 95 and SH-aLRT values > 80 had strong support. Additionally, we inferred phylogenies from mithocondrial DNA and the nuclear gene RAG1 separately to compare the topology of the phylogenetic tree derived from DNA regions with independent segregation.

Morphology

Diagnostic characters and comparisons are based on preserved specimens from Museo de Zoología at Pontificia Universidad Católica del Ecuador, Quito (QCAZ) and, when available digital photographs. Examined specimens are listed as Suppl. material 1. Character definitions and terminology follow Duellman and Lehr (2009). For subarticular tubercle terminology we follow Ron et al. (2020). Sex was determined by presence of nuptial pads or vocal slits, and direct inspection of gonads. Descriptions of coloration and variation in life are based on digital photographs. We examined the following qualitative characters: dorsal and ventral skin texture, presence of tympanic membrane and annulus, snout shape, presence of rostral papilla, presence of vomerine odontophores, presence of vocal slits and gular sac in males, relative length of fingers and toes, disc shape, presence of dorsolateral, discoidal and supratympanic folds, presence of lateral fringes on fingers and toes, presence of palmar, ulnar, tarsal, metatarsal, subarticular, supernumerary, knee, heel, and eyelid tubercles, and webbing on fingers and toes. We follow the name "hyperdistal tubercle" proposed in Ospina-Sarria and Duellman (2019) to refer to the most distal tubercle in Fingers and Toes.

Adults were measured with digital calipers (to the nearest ± 0.01mm) for eleven morphological variables, following Duellman and Lehr (2009): (1) snout-vent length; (2) tibia length; (3) foot length; (4) head length;

Table 1. Genbank accession numbers for DNA sequences used for phylogenetic analyses.

Species	Voucher	12S	16S	RAG1	ND1
Niceforonia elassodisca	QCAZ52495	MW567328	MW567375	MW451754	MW567403
Niceforonia nigrovittata	QCAZ59410	NA	MW567340	NA	NA
Pristimantis acerus	KU 217786	EF493678.1	EF493678.1	NA	NA
P. actites	KU 217830	NA	EF493696.1	EF493432.1	NA
P. acuminatus	CORBIDI 4769	MN128391	NA	NA	NA
	QCAZ53263	MN128394	NA	NA	NA
	CORBIDI 7469	MN128392	NA	NA	NA
	CORBIDI 7579	MN128393	NA	NA	NA
P. aff. subsigillatus	QCAZ58017	MW567302	MW567373	MW451755	MW567411
P. altamazonicus	KU 215460	EF493670.1	EF493670.1	NA	NA
P. amaguanae	QCAZ39274	MT636506	MT636529	MT635622	MT635661
P. andinognomus	QCAZ16683	NA	MW567356	MW451748	MW567390
P. angustilineatus	UVC 15828	NA	JN371034.1	NA	NA
P. appendiculatus	KU177637	EF493524.1	EF493524.1	NA	NA
P. aureolineatus	QCAZ42286	MT636509	MT636530	MT635626	NA
P. bambu	QCAZ46708	NA	MW567357	MW451727	NA
P. boulengeri	MHUAA 8951	NA	KU724435.1	NA	NA
P. brevifrons	nrps 0059	JN991498.1	JN991433.1	NA	NA
P. bromeliaceus	QCAZ16699	MT636505	MT636527	MT635618	MT635659
	QCAZ62940	MT636512	MT636523	NA	MT635669
P. calcarulatus	KU 177658	EF493523.1	EF493523.1	NA	NA
P. cedros	MZUTI 1713	NA	KT210155.1	NA	NA
P. celator	QCAZ66230	MW567326	MW567368	MW451723	MW567406
P. cf. mendax	MTD 45080	EU186659.1	EU186659.1	NA	NA
P. conspicillatus	QCAZ28448	MW567327	MW567377	MW451749	NA
	QCAZ55439	NA	MW567358	MW451746	MW567394
P. crucifer	KU 177733	EU186736.1	EU186718.1	NA	NA
P. curtipes	QCAZ40722	MW567323	MW567363	MW451750	MW567416
P. degener	QCAZ40304	MW567305	MW567376	MW451761	MW567379
P. diadematus	KU 221999	EU186668.1	EU186668.1	NA	NA
	QCAZ59442	MW567325	MW567354	MW451735	MW567419
P. dissimulatus	KU179090	EF493522.1	EF493522.1	NA	NA
P. dorsopictus	MHUAA7638	KP082864.1	KP082874.1	NA	NA
P. ecuadorensis	CJ 5350	KX785339	KX785343	NA	KX785347
	CJ 5351	KX785340	KX785344	NA	KX785348
P. enigmaticus	QCAZ40918	MT636513	MT636520	MT635636	MT635670
P. eremitus	QCAZ40002	NA	NA	MW451729	MW567391
	QCAZ49652	NA	MW567366	MW451732	MW567409
	QCAZ43392	NA	MW567333	NA	NA
P. eugeniae	DHMECN8809	MT853052	MT853039	NA	NA
	QCAZ52367	MW567322	MW567353	MW451743	MW567380
P. galdi	QCAZ32368	EU186670.1	EU186670.1	EU186746	NA
	QCAZ58885	MW567304	MW567355	MW451756	MW567410
	QCAZ58886	NA	MW567334	MW451757	NA
	QCAZ58888	NA	MW567335	MW451739	NA
P. glandulosus	KU 218002	EF493676.1	EF493676.1	NA	NA
P. imitatrix	KU 215476	EF493824.1	EF493667.1	NA	NA
P. inusitatus	KU 218015	EF493677.1	NA	NA	NA
P. jaguensis	MHUAA 7249	KP082862.1	KP082870.1	NA	NA
P. katoptroides	QCAZ46360	NA	MW567378	MW451753	MW567408
	QCAZ58897	NA	MW567330	NA	NA
P. lacrimosus	QCAZ55238	NA	MT636518	MT635629	MT635667
	QCAZ59474	NA	MT636517	MT635633	NA
	QCAZ40261	NA	MT636524	MT635623	MT635671
	QCAZ59469	NA	MT636516	MT635632	NA
P. limoncochensis	QCAZ43794	NA	MT636525	MT635627	MT635665
	QCAZ52987	MN128397	NA	NA	NA
	QCAZ56316	MN128398	NA	NA	NA
	QCAZ37277	MN128396	NA	NA	NA
	QCAZ19180	MN128395	MT636532	MT635620	NA
P. lymani	QCAZ46311	NA	MW567365	MW451752	NA
P. melanogaster	NA	EF493826.1	EF493664.1	NA	NA
P. mindo	MZUTI 1382	NA	KF801584.1	NA	NA
	MZUTI 1381	NA	KF801583.1	NA	NA
	QCAZ56512	NA	MT636522	MT635630	MT635668
	MZUTI 1756	NA	KF801581.1	NA	NA
	QCAZ42197	MT636508	MT636531	MT635625	MT635664
P. moro	AJC 1860	JN991520.1	JN991454.1	JQ025191.1	NA
	AJC 1753	JN991519.1	JN991453.1	JQ025192.1	NA
P. muranunka	QCAZ54593	NA	MW567358	MW451762	MW567393

Species	Voucher	12S	16S	RAG1	ND1
P. nankints	QCAZ69137	NA	MT636514	MT635635	NA
P. nyctophylax	KU 177812	EF493526.1	EF493526.1	NA	NA
	QCAZ32288	NA	MT636519	MT635621	MT635660
	KU 218147	EF493525.1	EF493525.1	NA	NA
	QCAZ49637	NA	MT636521	MT635628	MT635666
P. olivaceus	CORBIDI 17473	NA	KX155579	NA	NA
P. omeviridis	QCAZ55392	MN128401	NA	NA	NA
	FHGO 7192	MN128399	NA	NA	NA
	QCAZ10564	MN128400	MK881398	MK881312	MT635658
	QCAZ19664	NA	EU13057	MT635619	NA
P. orcesi	KU 218021	EF493679.1	EF493679.1	NA	NA
P. ornatissimus	MZUTI 4798	KU720464	KU720463	NA	KU720480
	MZUTI 4806	KX785337	KX785341	NA	KX785345
	MZUTI 4807	KX785338	KX785342	NA	KX785346
P. pahuma	MZUTI 493	NA	KT210158.1	NA	NA
P. petersi	QCAZ63455	MW567303	MW567369	MW451725	MW567405
	QCAZ51390	NA	MZ219640	MZ219636	MZ219631
	QCAZ15988	NA	MZ219641	MZ219635	MZ219633
	QCAZ63456	NA	MW567367	MW451726	MW567404
P. petersioides sp. nov.	QCAZ58936	NA	MW567331	MW451740	MW567401
	QCAZ58937	MW567306	MW567336	MW451758	MW567381
	QCAZ58951	MW567321	MW567339	MW451741	MW567389
	QCAZ58939	MW567307	MW567337	MW451759	MW567386
	QCAZ59167	MW567309	MW567332	MW451763	MW567387
	QCAZ58944	MW567308	MW567338	MW451760	MW567395
	QCAZ59456	MW567312	MW567352	MW451744	MW567388
	QCAZ59466	MW567314	MW567343	MW451745	MW567383
	QCAZ59472	MW567317	MW567347	MW451719	MW567382
	QCAZ59479	MW567318	MW567348	MW451724	MW567384
	QCAZ59625	MW567319	MW567349	MW451720	MW567385
	QCAZ59470	MW567316	MW567345	MW451737	MW567397
	QCAZ59471	MW567311	MW567346	MW451738	MW567420
	QCAZ59468	MW567315	MW567344	MW451742	MW567402
	QCAZ59458	MW567310	MW567341	MW451736	MW567395
	QCAZ59461	MW567313	MW567342	MW451718	MW567398
	QCAZ49027	NA	MZ219642	MZ219638	MZ219634
P. platydactylus	MNCNDNA 5524	FJ438811.1	EU192255.1	NA	NA
P. pluvialis	CORBIDI 11862	NA	KX155577	NA	NA
	CORBIDI 16695	NA	KX155578	NA	NA
P. pulchridormientes	CORBIDI 15563	NA	KX664106	NA	NA
	CORBIDI 15566	NA	KX664107	NA	NA
P. pulvinatus	KU 181015	EF186741.1	EF186723.1	NA	NA
P. pycnodermis	KU 218028	EF493680.1	EF493680.1	NA	NA
P. quaquaversus	QCAZ25613	MW567329	MW567360	MW451722	MW567407
P. romeroae	QCAZ41121	MT636507	MT636528	MT635624	MT635662
P. rubicundus	QCAZ58932	NA	MT372670	MT372613	NA
	KU 212220	EF493681.1	EF493681.1	NA	NA
P. schultei	KU 291702	EF493351.1	EF493351.1	NA	NA
Pristimantis sp.	QCAZ63481	NA	MW567350	NA	MW567399
	QCAZ63482	NA	MW567351	MW451721	MW567400
	ROM 43978	EU186678.1	EU186678.1	NA	NA
	QCAZ60398	NA	MT636515	MT635634	NA
	QCAZ58956	MT636511	MT636526	MT635631	NA
	CORBIDI 13805	NA	MZ219639	MZ219637	MZ219632
	MECN 10117	NA	KF801580.1	NA	NA
P. subsigillatus	QCAZ45268	NA	MW567370	MW451730	MW567412
	QCAZ49370	MW567320	MW567371	MW451731	MW567413
	QCAZ51314	NA	MW567361	MW451734	MW567392
	QCAZ50012	NA	MW567372	MW451733	MW567414
	MHNC 12845	MN128403	NA	NA	NA
P. tantanti	CORBIDI 12987	MN128402	NA	NA	NA
P. truebae	QCAZ13752	NA	MW567362	MW451747	MW567417
	QCAZ42714	MW567324	MW567364	MW451751	MW567418
P. urani	MHUAA 7471	NA	KU724442.1	NA	NA
P. w-nigrum	QCAZ45200	MT636510	MT372703	MT372600	MT372569
	QCAZ46256	NA	MT372704	MT372603	MT372571
	QCAZ41818	NA	MT372691	NA	MT635663
	QCAZ52365	NA	MW567374	MW451728	MW567415
P. zorro	MHUAA 8813	NA	MT747833	NA	NA
	MHUAA 8814	NA	MT747834	NA	NA
	MHUAA 8816	NA	MT747835	NA	NA

(5) head width; (6) eye diameter; (7) tympanum diameter; (8) interorbital distance; (9) eye width; (10) internarial distance; and (11) eye-nostril distance. Morphometric analyses were performed based on measurements of adults (number of specimens in parenthesis): *P. petersi* (12), *P.* aff. *petersi* (new species; 54).

To explore morphometric differentiation between species, we applied a Principal Components Analysis (PCA). To remove the effect of size covariation, we carried out linear regressions between the morphometric variables and SVL. The PCA was applied to the residuals of the regressions. Morphometric variables associated with eyes (i.e., eye diameter, interorbital distance, eyelid width, internarial distance and eye-nostril distance) had weak correlation with snout-vent length. Low correlation appears to be a result of the difficulty of defining the eye edge on preserved specimens. Therefore, were removed those variables from the analysis. Prior to the PCA, we ran a MANOVA on the residuals to test for morphometric sexual dimorphism independent of size differences . Because the MANOVA was non-significant, we pooled the measurements of both sexes on a single PCA.

Bioacoustics

To assess species limits between the new species and the closely related *P. petersi*, we analyzed calls from three males of the new species: QCAZ58940, SVL = 19.3 mm, from Refuge 1, Sardinayacu, Sangay National Park, Morona Santiago Province (2.0983°S, 78.1555°W, 1406 m) collected on 21 January 2015, air temperature 19 °C, recorded *in situ* by Daniel Rivadeneira, and QCAZ59466, SVL = 19.1 mm, from the ravines of Yurugyacu river, Zarentza community, Llanganates National Park, Pastaza Province, (1.3524°S, 78.0597°W, 1419 m) collected on February 24 2015 and recorded in captivity on 6 March 2015 by Santiago R. Ron and one male not collected from Sardinayacu, Sangay National Park, Morona Santiago Province, recorded by Diego Batallas. Advertisement calls of *P. petersi* were analyzed from two adult males (not collected) from near its type locality, Cocodrilos, Napo Province, (0.66812°S, 77.7975°W, 1725 m) recorded on 22 June 2016 by Santiago R. Ron. We did not have size data for the recorded individuals of *P. petersi*. However, we collected other adult males on the same night and chorus and used the size of those individuals to assess interpopulation size differences. For two of the recorded males of the new species, SVL was 19.1 and 19.3 mm; for *Pristimantis petersi* average SVL was 17.2 mm (16.5–17.8 mm; $n = 3$). Recordings were made in WAV format, with a sample rate of 44100 Hz and 16-bits. Call variables were measured with RAVEN PRO 1.5 (Charif et al. 2010), under a Hanning function, 2048 DFT, sample rate of 46 kHz and a grid spacing of 20 kHz.

Most of our recordings lacked temperature information. However, the variables that allowed us to differentiate the new species from the closely related *P. petersi*, where call duration and call frequency which are static and are not strongly influenced by ambient temperature (Köhler et al. 2017). Moreover, equatorial Andean forests have low seasonality and with low temperature variation, so it is unlikely that our results are biased by temperature differences between localities or seasons. At Zarentza, for example, in 2015, the monthly average of the minimum daily temperature varied between 14.7 (January) and 15.9 °C (May) while at Cocodrilos (where recordings for *P. petersi* were made) it had a range between 15.1 (January) and 16.0 °C (November). Temperature data were obtained from the WorldClim database (https://www.worldclim.org/).

For call measurements and terminology, we followed the call-centered approach by Köhler et al. (2017). We followed the step-by-step guide of Köhler et al. (2017) for measuring variables: we measured temporal variables such as call duration, call rate, call interval, call rise time and amplitude modulation in the oscillogram and the spectral variables such as frequency band, fundamental frequency, and dominant frequency in the spectrogram. Due to call structure, parameters such as notes, notes rates, note series, pulses and pulse rates were absent and only eight acoustic parameters (modified from Köhler et al. 2017) were measured: (1) Call duration = time from beginning to end of the call, measured from oscillogram; (2) Call rate = number of calls per minute; (3) Call interval = time from end of call to beginning of next call;(4) Call rise time = time from beginning of call to point of maximum amplitude; (5) Amplitude modulation = change in the amplitude level of a sound wave over time; (6) Frequency band = difference between upper and lower frequencies measured visually along the entire call (7) Fundamental frequency = frequency with highest energy on 1st harmonic in the call; (8) Dominant frequency = frequency with highest energy along entire call. Recordings are deposited in the Sound Archive of Museo de Zoología QCAZof Pontificia Universidad Católica del Ecuador and are available at the Anfibios del Ecuador website, https://bioweb.bio/faunaweb/amphibiaweb/).

Results

Phylogeny and genetic distances

The Maximum Likelihood tree (Fig. 1) is similar in topology to Rivera-Correa and Daza (2016), Rivera-Correa and Daza (2020), and Ron et al. (2020). Support values for the *Pristimantis lacrimosus* group is strong (bootstrap = 100); this clade includes the species reported in Ron et al. (2020) as well as species not included in previous phylogenies as *P. degener* (Lynch and Duellman, 1997), *P. petersi*, and the new species. Additionally, we report for the first time the phylogenetic position of *Pristimantis eugeniae* (Lynch and Duellman, 1997) and *P. katoptroides* (Flores, 1988). *Pristimantis eugeniae* is the sister species of a clade formed by *P. glandulosus* (Boulenger,

1880), *P. acerus* (Lynch and Duellman, 1980), and *P. inusitatus* (Lynch and Duellman, 1980) (bootstrap = 99). *Pristimantis katoptroides* is sister to *P. quaquaversus* (Lynch, 1974) + *P. melanogaster* (Duellman and Pramuk, 1999). *Pristimantis subsigillatus* (Boulenger, 1902) was polyphyletic because two samples (KU 218147 and QCAZ49637) were more closely related to *P. nyctophylax* (Lynch, 1976) than to the other samples of *P. subsigillatus*. After reviewing the morphology of QCAZ49637 we concluded that it belongs to *P. nyctophylax*. Because KU 218147 is genetically identical, we also assigned it to *P. nyctophylax*.

The ML tree from mitochondrial DNA shows similar topology to the ML tree from all genes. The best-fit models of DNA evolution for each partition are available as Suppl. material 2.

The mtDNA tree shows strong support for the *Pristimantis lacrimosus* group (bootstrap = 99), for *Pristimantis petersi* (bootstrap = 100) and for the new species (bootstrap = 99). The ML tree inferred from RAG1 shows lower support values but is congruent in showing a monophyletic *Pristimantis lacrimosus* group (bootstrap = 91) and in confirming a close relationship between *P. petersi* and the new species. The mtDNA and RAG1 phylogenies do not show strongly supported incongruences. Mitochondrial DNA and RAG1 phylogenetic trees are available as Suppl. material 3.

The new species is the sister to *P. petersi* and an undescribed species from Cordillera Escalera, Peru. The uncorrected pairwise *p*-genetic distances for 16S between *P. petersi* and the new species range from 7.9% to 8.4%. The clade comprising these three species is sister to an

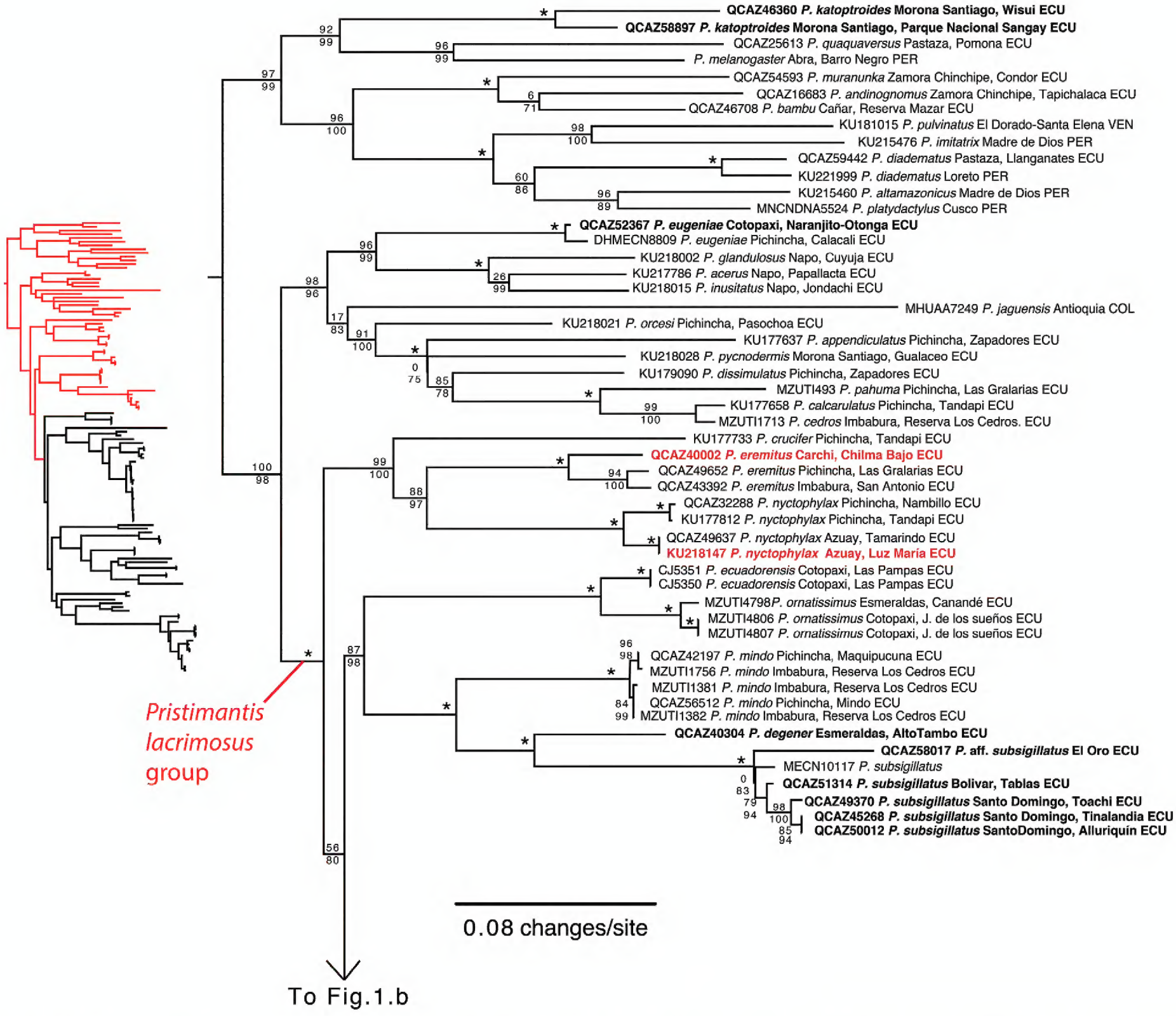
QCAZ46360 P. katoptroides Morona Santiago, Wisui ECU
QCAZ58897 P. katoptroides Morona Santiago, Parque Nacional Sangay ECU
QCAZ25613 P. quaquaversus Pastaza, Pomona ECU
P. melanogaster Abra, Barro Negro PER
QCAZ54593 P. muranunka Zamora Chinchipe, Condor ECU
QCAZ16683 P. andinognomus Zamora Chinchipe, Tapichalaca ECU
QCAZ46708 P. bambu Cañar, Reserva Mazar ECU
KU181015 P. pulvinatus El Dorado-Santa Elena VEN
KU215476 P. imitatrix Madre de Dios PER
QCAZ59442 P. diadematus Pastaza, Llanganates ECU
KU221999 P. diadematus Loreto PER
KU215460 P. altamazonicus Madre de Dios PER
MNCNDNA5524 P. platydactylus Cusco PER
QCAZ52367 P. eugeniae Cotopaxi, Naranjito-Otonga ECU
DHMECN8809 P. eugeniae Pichincha, Calacali ECU
KU218002 P. glandulosus Napo, Cuyuja ECU
KU217786 P. acerus Napo, Papallacta ECU
KU218015 P. inusitatus Napo, Jondachi ECU
MHUAA7249 P. jaguensis Antioquia COL
KU218021 P. orcesi Pichincha, Pasochoa ECU
KU177637 P. appendiculatus Pichincha, Zapadores ECU
KU218028 P. pycnodermis Morona Santiago, Gualaceo ECU
KU179090 P. dissimulatus Pichincha, Zapadores ECU
MZUTI493 P. pahuma Pichincha, Las Gralarias ECU
KU177658 P. calcarulatus Pichincha, Tandapi ECU
MZUTI1713 P. cedros Imbabura, Reserva Los Cedros. ECU
KU177733 P. crucifer Pichincha, Tandapi ECU
QCAZ40002 P. eremitus Carchi, Chilma Bajo ECU
QCAZ49652 P. eremitus Pichincha, Las Gralarias ECU
QCAZ43392 P. eremitus Imbabura, San Antonio ECU
QCAZ32288 P. nyctophylax Pichincha, Nambillo ECU
KU177812 P. nyctophylax Pichincha, Tandapi ECU
QCAZ49637 P. nyctophylax Azuay, Tamarindo ECU
KU218147 P. nyctophylax Azuay, Luz María ECU
CJ5351 P. ecuadorensis Cotopaxi, Las Pampas ECU
CJ5350 P. ecuadorensis Cotopaxi, Las Pampas ECU
MZUTI4798P. ornatissimus Esmeraldas, Canandé ECU
MZUTI4806 P. ornatissimus Cotopaxi, J. de los sueños ECU
MZUTI4807 P. ornatissimus Cotopaxi, J. de los sueños ECU
QCAZ42197 P. mindo Pichincha, Maquipucuna ECU
MZUTI1756 P. mindo Imbabura, Reserva Los Cedros ECU
MZUTI1381 P. mindo Imbabura, Reserva Los Cedros ECU
QCAZ56512 P. mindo Pichincha, Mindo ECU
MZUTI1382 P. mindo Imbabura, Reserva Los Cedros ECU
QCAZ40304 P. degener Esmeraldas, AltoTambo ECU
QCAZ58017 P. aff. subsigillatus El Oro ECU
MECN10117 P. subsigillatus
QCAZ51314 P. subsigillatus Bolivar, Tablas ECU
QCAZ49370 P. subsigillatus Santo Domingo, Toachi ECU
QCAZ45268 P. subsigillatus Santo Domingo, Tinalandia ECU
QCAZ50012 P. subsigillatus SantoDomingo, Alluriquín ECU
Pristimantis lacrimosus group
0.08 changes/site
To Fig.1.b

Figure 1. Maximum likelihood tree of the *Pristimantis lacrimosus* group inferred from a partitioned analysis of 4026 aligned sites of DNA sequences of the mitochondrial genes 12S, 16S, and ND-1 and the nuclear gene RAG-**1.** Bold characters highlight individuals included for the first time in a phylogeny, red taxa highlight previously misidentified species. *Pristimantis petersioides* sp. nov. is highlighted in blue. SH-aLRT support (above branch) and ultrafast bootstrap support (below) are shown as percentages; asterisks denote 100% for both measures. Outgroups are not shown. Number for voucher museum specimens are shown to the left of the species name; locality is shown to the right and country abbreviation at the end, as follows: **ECU** Ecuador, **PER** Peru, **COL** Colombia, **VEN** Venezuela, **GUY** Guyana, **PAN** Panama.

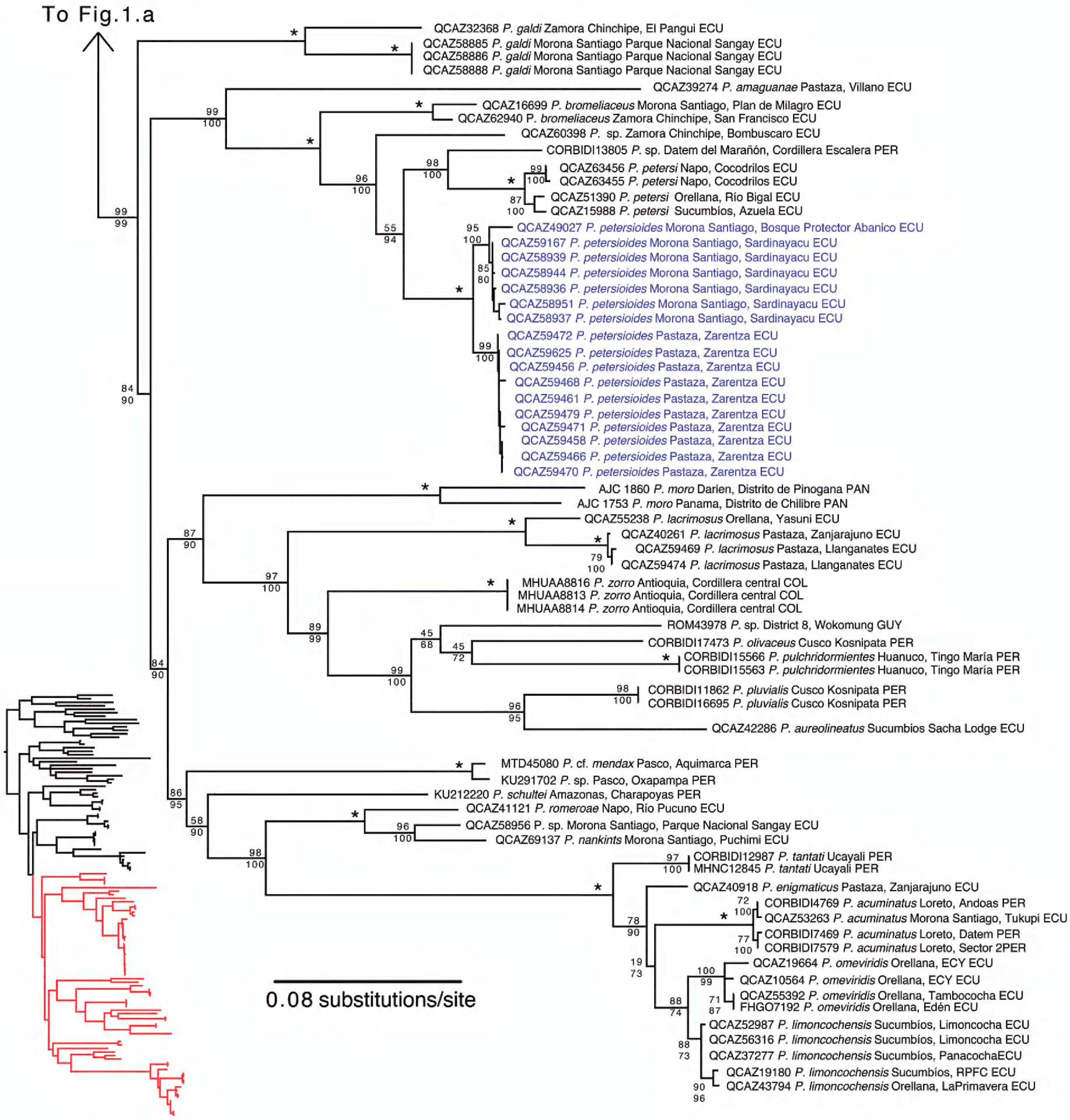
To Fig.1.a
QCAZ32368 P. galdi Zamora Chinchipe, El Pangui ECU
QCAZ58885 P. galdi Morona Santiago Parque Nacional Sangay ECU
QCAZ58886 P. galdi Morona Santiago Parque Nacional Sangay ECU
QCAZ58888 P. galdi Morona Santiago Parque Nacional Sangay ECU
QCAZ39274 P. amaguanae Pastaza, Villano ECU
QCAZ16699 P. bromeliaceus Morona Santiago, Plan de Milagro ECU
QCAZ62940 P. bromeliaceus Zamora Chinchipe, San Francisco ECU
QCAZ60398 P. sp. Zamora Chinchipe, Bombuscaro ECU
CORBIDI13805 P. sp. Datem del Marañón, Cordillera Escalera PER
QCAZ63456 P. petersi Napo, Cocodrilos ECU
QCAZ63455 P. petersi Napo, Cocodrilos ECU
QCAZ51390 P. petersi Orellana, Río Bigal ECU
QCAZ15988 P. petersi Sucumbíos, Azuela ECU
QCAZ49027 P. petersioides Morona Santiago, Bosque Protector Abanico ECU
QCAZ59167 P. petersioides Morona Santiago, Sardinayacu ECU
QCAZ58939 P. petersioides Morona Santiago, Sardinayacu ECU
QCAZ58944 P. petersioides Morona Santiago, Sardinayacu ECU
QCAZ58936 P. petersioides Morona Santiago, Sardinayacu ECU
QCAZ58951 P. petersioides Morona Santiago, Sardinayacu ECU
QCAZ58937 P. petersioides Morona Santiago, Sardinayacu ECU
QCAZ59472 P. petersioides Pastaza, Zarentza ECU
QCAZ59625 P. petersioides Pastaza, Zarentza ECU
QCAZ59456 P. petersioides Pastaza, Zarentza ECU
QCAZ59468 P. petersioides Pastaza, Zarentza ECU
QCAZ59461 P. petersioides Pastaza, Zarentza ECU
QCAZ59479 P. petersioides Pastaza, Zarentza ECU
QCAZ59471 P. petersioides Pastaza, Zarentza ECU
QCAZ59458 P. petersioides Pastaza, Zarentza ECU
QCAZ59466 P. petersioides Pastaza, Zarentza ECU
QCAZ59470 P. petersioides Pastaza, Zarentza ECU
AJC 1860 P. moro Darien, Distrito de Pinogana PAN
AJC 1753 P. moro Panama, Distrito de Chilibre PAN
QCAZ55238 P. lacrimosus Orellana, Yasuni ECU
QCAZ40261 P. lacrimosus Pastaza, Zanjarajuno ECU
QCAZ59469 P. lacrimosus Pastaza, Llanganates ECU
QCAZ59474 P. lacrimosus Pastaza, Llanganates ECU
MHUAA8816 P. zorro Antioquia, Cordillera central COL
MHUAA8813 P. zorro Antioquia, Cordillera central COL
MHUAA8814 P. zorro Antioquia, Cordillera central COL
ROM43978 P. sp. District 8, Wokomung GUY
CORBIDI17473 P. olivaceus Cusco Kosnipata PER
CORBIDI15566 P. pulchridormientes Huanuco, Tingo María PER
CORBIDI15563 P. pulchridormientes Huanuco, Tingo María PER
CORBIDI11862 P. pluvialis Cusco Kosnipata PER
CORBIDI16695 P. pluvialis Cusco Kosnipata PER
QCAZ42286 P. aureolineatus Sucumbios Sacha Lodge ECU
MTD45080 P. cf. mendax Pasco, Aquimarca PER
KU291702 P. sp. Pasco, Oxapampa PER
KU212220 P. schultei Amazonas, Charapoyas PER
QCAZ41121 P. romeroae Napo, Río Pucuno ECU
QCAZ58956 P. sp. Morona Santiago, Parque Nacional Sangay ECU
QCAZ69137 P. nankints Morona Santiago, Puchimi ECU
CORBIDI12987 P. tantati Ucayali PER
MHNC12845 P. tantati Ucayali PER
QCAZ40918 P. enigmaticus Pastaza, Zanjarajuno ECU
CORBIDI4769 P. acuminatus Loreto, Andoas PER
QCAZ53263 P. acuminatus Morona Santiago, Tukupi ECU
CORBIDI7469 P. acuminatus Loreto, Datem PER
CORBIDI7579 P. acuminatus Loreto, Sector 2PER
QCAZ19664 P. omeviridis Orellana, ECY ECU
QCAZ10564 P. omeviridis Orellana, ECY ECU
QCAZ55392 P. omeviridis Orellana, Tambococha ECU
FHGO7192 P. omeviridis Orellana, Edén ECU
QCAZ52987 P. limoncochensis Sucumbíos, Limoncocha ECU
QCAZ56316 P. limoncochensis Sucumbíos, Limoncocha ECU
QCAZ37277 P. limoncochensis Sucumbíos, PanacochaECU
QCAZ19180 P. limoncochensis Sucumbíos, RPFC ECU
QCAZ43794 P. limoncochensis Orellana, LaPrimavera ECU
0.08 substitutions/site

Figure 1. Continued.

undescribed species from Bombuscaro, Podocarpus National Park, Zamora Province, Ecuador. Samples of the new species separate in two sister clades, a northern clade in Zarentza, Pastaza Province and a southern clade from Sardinayacu and Bosque Protector Abanico, Morona Santiago Province; the uncorrected pairwise *p*-genetic distances between these clades range from 1.2% to 1.4%.

Morphometric analysis

MANOVA results showed no sexual dimorphism (after removing size effects) on the new species and *P. petersi*. The PCA show broad overlap in morphometric space between both species (Fig. 2). PC I (49.5%) had high loadings on head width, head length, and tibia length while PC II (21.5%) had high loading on tympanum diameter. Both principal components explained 71.0% of the morphometric variation (Table 2).

Systematic account

The differences in advertisement calls (see Comparisons with other Species section), the branch lengths in the phylogeny, and genetic distances indicate that the new species, in fact, represents a different species from *P. petersi* (see below). In the following section, we update the species content of the *Pristimantis lacrimosus* group and describe the new species.

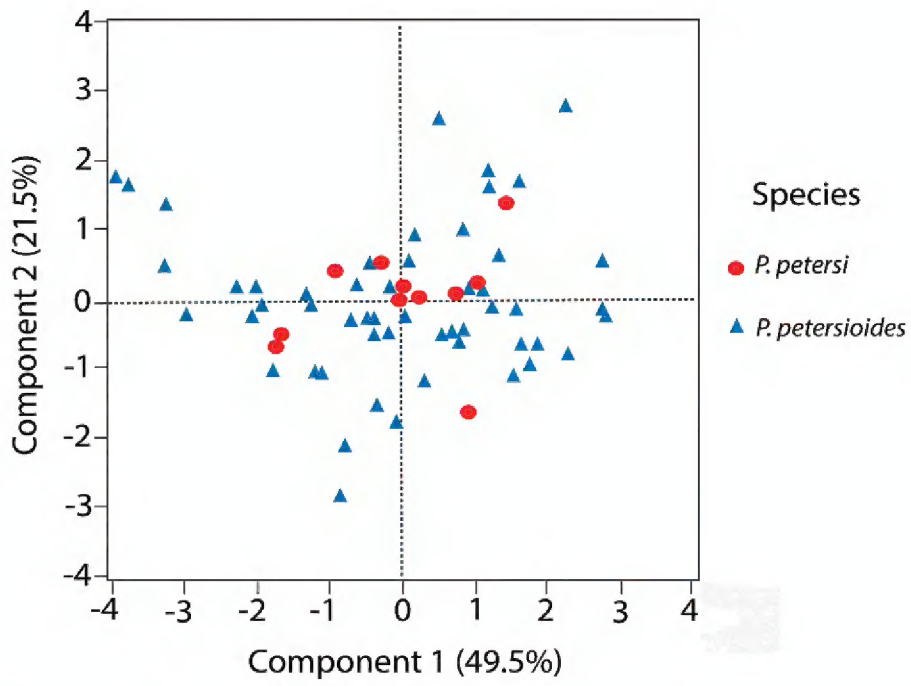

Figure 2. Principal components 1 and 2 from analysis of five size-corrected morphological variables. See Table 2 for character loadings on each component.

Table 2. Character loadings, eigenvalues, and percentage of explained variance for Principal Components (PC) I–II. The analysis was based on the residuals of morphometric variables of adult *P. petersioides* sp. nov. and *P. petersi*. Bold figures indicate highest loadings.

Variable	Character loading	
	PC I	PC II
Tibia length	**0.7855**	-0.3038
Foot length	0.7066	0.0834
Head length	**0.7873**	0.2181
Head width	**0.8332**	0.2434
Tympanum	-0.2087	**0.9334**
Eigenvalues	4.9483	2.1547
%	49.48	21.54

Pristimantis lacrimosus species group

Content. We include all the descendants from the most recent common ancestor of *P. eremitus* and *P. lacrimosus* according to Ron et al. 2020. We exclude *Pristimantis eugeniae* from this group because it belongs to the sister clade of *P. lacrimosus* species group. We also include *P. degener* which is sister to *P. subsigillatus* and the new species.

According to our findings and those of Ron et al. 2020, the *Pristimantis lacrimosus* group comprises 39 species (species included in the phylogeny are marked with an asterisk): **P. acuminatus* (Shreve, 1935), **P. amaguanae* Ron, Carrión, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, **P. aureolineatus* (Guayasamin, Ron, Cisneros-Heredia, Lamar & McCracken, 2006), **P. bromeliaceus* (Lynch, 1979), *P. calima* Ospina-Sarria & Duellman, 2019, **P. crucifer* (Boulenger, 1899), **P. degener* (Lynch & Duellman, 1997), *P. deyi* Lehr, Gregory & Catenazzi, 2013, **P. ecuadorensis* Guayasamin, Hutter, Tapia, Culebras, Peñafiel, Pyron, Morochz, Funk & Arteaga-Navarro, 2017, **P. enigmaticus* Ortega-Andrade, Rojas-Soto, Valencia, Espinosa de los Monteros, Morrone, Ron & Cannatella, 2015, **P. eremitus* (Lynch, 1980), **P. galdi* Jiménez de la Espada, 1870, **P. lacrimosus* (Jiménez de la Espada, 1875), *P. latericius* Batallas & Brito, 2014, **P. limoncochensis* Ortega-Andrade, Rojas-Soto, Valencia, Espinosa de los Monteros, Morrone, Ron & Cannatella, 2015, **P. mendax* (Duellman, 1978), **P. mindo* Arteaga, Yanez-Munoz & Guayasamin, 2013, **P. moro* (Savage, 1965), **P. nankints* Ron, Carrión, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, **P. nyctophylax* (Lynch ,1976), **P. olivaceus* (Köhler, Morales, Lötters, Reichle & Aparicio, 1998), **P. omeviridis* Ortega-Andrade, Rojas-Soto, Valencia, Espinosa de los Monteros, Morrone, Ron & Cannatella, 2015, **P. ornatissimus* (Despax, 1911), *P. padiali* Moravec, Lehr, Pérez-Peña, López, Gagliardi-Urrutia & Arista-Tuanama, 2010, *P. pardalinus* (Lehr, Lundberg, Aguilar and von May, 2006), **P. petersi* (Lynch & Duellman, 1980), **P. petersioides* sp. nov. (herein), **P. pluvialis* Shepack, von May, Ttito, & Catenazzi, 2016, *P. pseudoacuminatus* (Shreve, 1935), **P. romeroae* Ron, Carrión, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, *P. royi* (Morales, 2007), **P. pulchridormientes* Chávez & Catenazzi, 2016, **P. schultei* (Duellman, 1990), **P. subsigillatus* (Boulenger, 1902), **P. tantanti* (Lehr, Torres-Gastello & Suárez-Segovia, 2007), *P. tayrona* (Lynch & Ruiz-Carranza, 1985), *P. waoranii* (McCracken, Forstner & Dixon, 2007), *P. zimmermanae* (Heyer & Hardy, 1991), and **P. zorro* Rivera-Correa & Daza 2020.

Distribution. The *Pristimantis lacrimosus* group is distributed in Central America, the Guianan Shield, Pacific Basin of Ecuador, and the Amazon Basin. Its species richness peaks in the Ecuadorian Andes (n = 19) and Amazon basin of Ecuador and Peru (n = 14).

Remarks. We refrain from assigning *Pristimantis sneiderni* (Ospina-Sarria and Duellman 2019) to the *Pristimantis lacrimosus* group due to the lack of molecular evidence and following Ron et al. (2020).

Pristimantis petersioides sp. nov.

http://zoobank.org/F2204639-4B59-4071-A033-5BE637144BEE

Eleutherodactylus petersi Lynch & Duellman 1980 (in part)
Pristimantis petersi Batallas & Brito 2016
Pristimantis petersi Brito et al. 2017

Holotype. (Figs 3, 4) QCAZ58939, adult female from Ecuador, Morona Santiago Province, Sangay National Park, Sardinayacu (2.0983°S, 78.1555°W), 1406 m. Found in amplexus with QCAZ58940. collected by Daniel Rivadeneira and Santiago R. Ron on 21 January 2015.

Paratypes (54: 39 adult males, 15 adult females). All individuals are adults unless otherwise noticed. All from Ecuador. *Morona Santiago Province*: Sangay National Park: QCAZ58871, female, QCAZ58944, male from Río Volcán (2.1008°S, 78.1559°W, 1345 m), collected by Daniel Rivadeneira, David Velalcázar, Javier Pinto, Francy Mora, Darwin Nuñez, Juan Sanchez, and Andrea Correa; QCAZ58936, 58939, QCAZ58941, females, QCAZ58940, 58942–43, males from Refuge

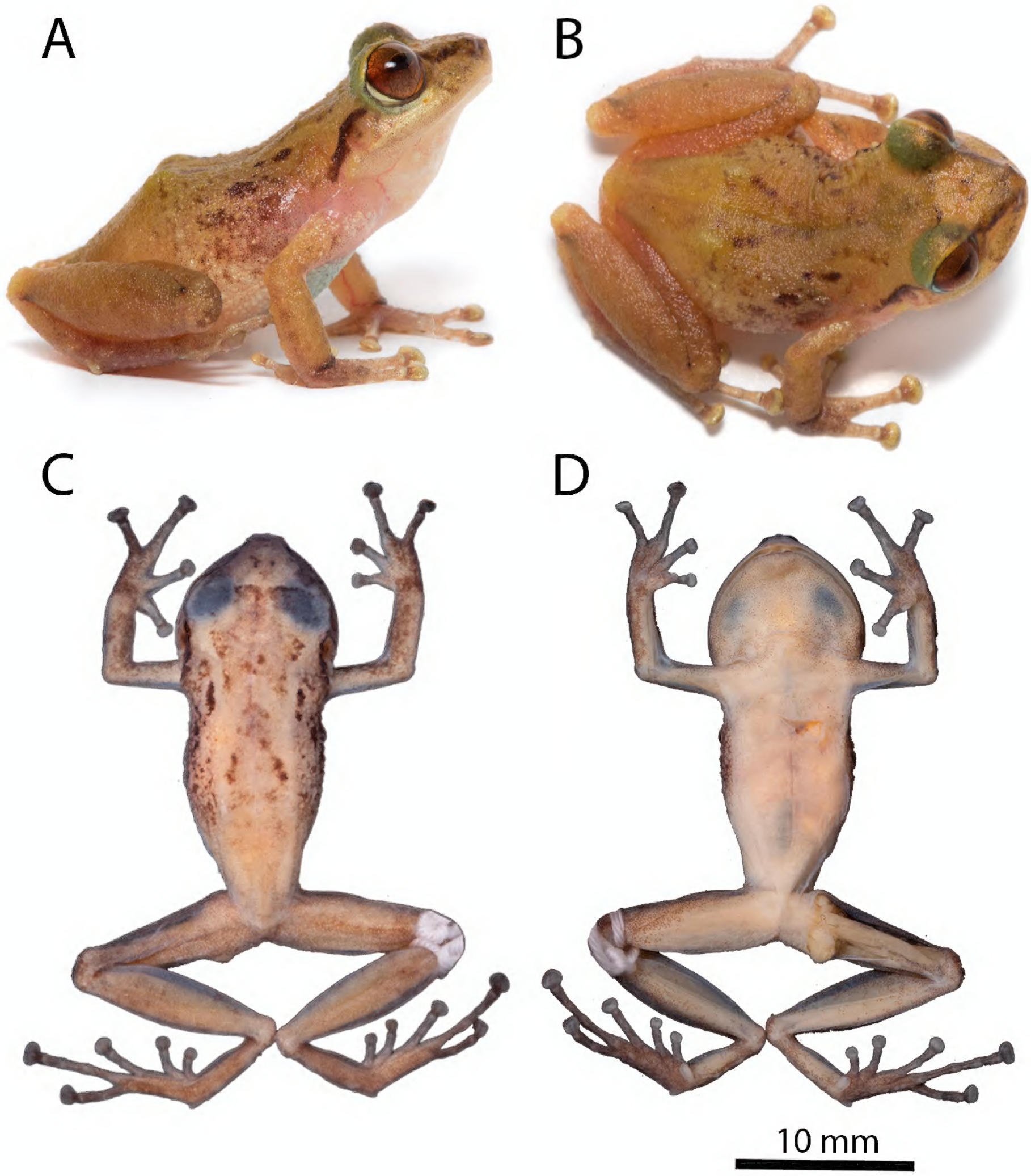

Figure 3. Holotype of *Pristimantis petersioides* sp. nov. QCAZ58939, adult female, SVL = 22.02 mm. Sangay National Park, Sardinayacu, Ecuador. **A** lateral view of live individual, **B** dorsal view of live individual, **C** dorsal view of preserved individual, **D** ventral view of preserved individual. Photographs **A, B** by Juan Carlos Sánchez, **C, D** by Julio C. Carrión-Olmedo

1 (2.0988°S, 78.1561°W, 1406 m), QCAZ58937–38, males from Chimerella lagoon (2.0885°S, 78.2069°W, 1650 m) collected by Daniel Rivadeneira, Francy Mora, Juan Sánchez and Andrea Correa; QCAZ58881, 58950, females, QCAZ58949 male from the proximities of Cormorant lagoon (2.0738°S, 78.2195°W, 1835 m) collected by Javier Pinto, David Velalcázar and Darwin Nuñez, QCAZ58880, 58951, males from El Enmascarado lagoon (2.0600°S, 78.2207°W, 1796 m) collected by Javier Pinto, David Velalcázar and Darwin Nuñez. in January 2015. QCAZ59166, female, QCAZ59167, 58945–48, males from Refuge 3 (2.0757°S, 78.2157°W, 1724 m), collected by Santiago Ron, Diego Paucar, Pablo Venegas, Pamela Baldeón, Marcel Caminer and Kunam Nucirquia; QCAZ59169–71, males from Cormorant lagoon (2.0738°S, 78.2195°W, 1835 m), collected by Santiago Ron, Diego Paucar, Pablo Venegas, Pamela Baldeón, Marcel Caminer and Kunam Nucirquia, in February 2015. *Pastaza Province*: QCAZ53227, female, from Anzu river (1.4177°S, 78.0485°W, 1272 m a.s.l), col-

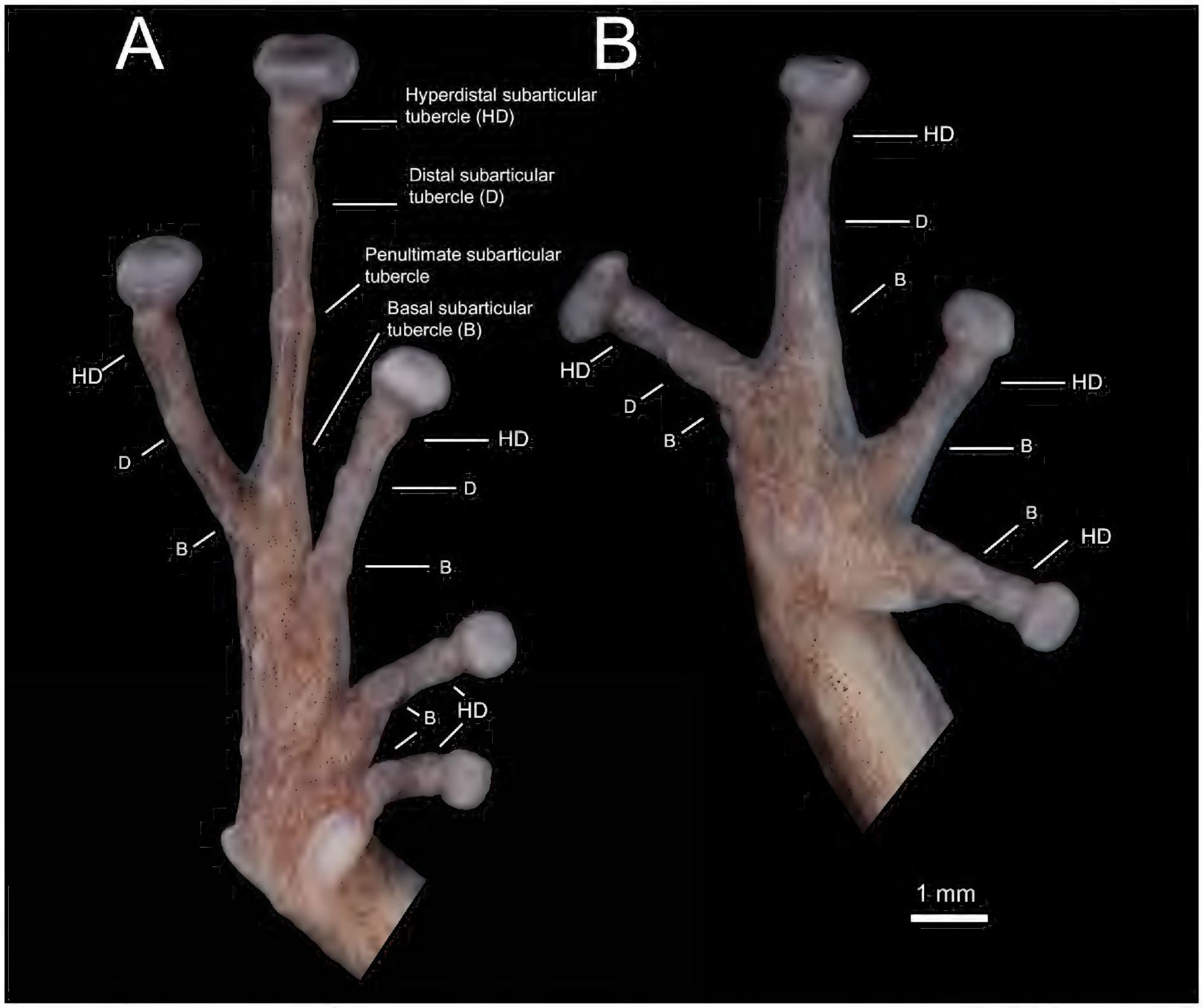

Figure 4. Hand and foot of the holotype of *Pristimantis petersioides* sp. nov. QCAZ58939, adult female. Ventral views of right foot **A** and right hand **B**. Abbreviations: B = basal, D = distal, HD = hyperdistal. Photographs by Julio C. Carrión-Olmedo.

lected by Mauricio Ortega in May 2012. Llanganates National Park: QCAZ45846–50, 45892, 45898, males, from Challuwa Yacu river, Ankaku Reserve (1.2792°S, 78.0779°W, 2300 m) collected by Elicio Tapia and Silvia Aldás in October 2009; QCAZ66553, male, from Ankaku Reserve (1.2770°S, 78.0698°W, 2216 m) collected by Diego Almeida, Santiago Guamán, Darwin Nuñez, María Navarrete, Verónica Andrade, Angel Alvarado, Fernando Alvarado in January 2017, QCAZ59625, male, from Nuchimingue river (1.3626°S, 78.0582°W, 1350 m); QCAZ59456, male, from Yurugyacu river (1.3560°S, 78.0592°W, 1354 m); QCAZ59451, 59467–68, 59479, males, from Zarentza community (1.3556°S, 78.0597°W, 1363 m); QCAZ59458–59, females from near Yurugyacu river (1.3527°S, 78.0596°W, 1354 m); QCAZ59457, 59465 females, QCAZ59454–55, 59462–63, 59466, males from the ravines of Yurugyacu river (1.3523°S, 78.0597°W, 1419 m); QCAZ59470, 59472, females, QCAZ59471, 59473, males from Gustavo Ushpa house trail to Yurugyacu river (1.3430°S, 78.0574°W, 1221 m); QCAZ59461, female, from La paila waterfall (1.3397°S, 78.0594°W, 1360 m) collected by Daniel Rivadeneira, Francy Mora, Juan Carlos Sánchez, David Velalcázar, Darwin Nuñez and Javier Pinto in February 2015.

Referred specimens. *Napo Province*: QCAZ46159, male, from Salcedo-Tena highway, km 60 (0.9847°S, 78.1928°W, 2253 m), collected by Elicio Tapia and Fernando Núñez in November 2009. *Pastaza Province*: QCAZ59452–53, 59460, 59464, juveniles from the ravines of Yurugyacu river (1.3523°S, 78.0597°W, 1419 m) collected by Daniel Rivadeneira, Francy Mora, Juan Carlos Sánchez, David Velalcázar, Darwin Nuñez and Javier Pinto in February 2015.

Suggested common name. English: Sardinayacu's Rain Frog. Spanish: Cutín de Sardinayacu

Diagnosis. The assignment of the new species to the genus *Pristimantis* is based on the phylogeny (Fig. 1). *Pristimantis petersioides* sp. nov. is characterized by the following combination of characters: (1) Skin on dorsum smooth to shagreen with or without scattered small tubercles, head with or without one interorbital small tubercle, skin of venter shagreened to weakly areolate; discoidal fold present, ill-defined; dorsolateral folds absent; (2) tympanic membrane and tympanic annulus present, round, its

length 2/5 to 1/2 of eye diameter; its upper border weakly concealed by inconspicuous supratympanic fold; (3) snout rounded to truncate in dorsal view, truncate in lateral view, bearing a small rostral papilla; (4) interorbital space flat, broader than upper eyelid; upper eyelid with one distinct subconical tubercle surrounded by lower, indistinct rounded tubercles; cranial crests absent; (5) vomerine odontophores low to prominent, oblique, moderately separated, posteromedial to choanae; (6) males with prominent, subgular vocal sac and vocal slits; (7) first finger shorter than second; all fingers long, discs broadly expanded, rounded to truncate; all fingers bearing a hyperdistal tubercle (Fig. 4B); (8) fingers with narrow lateral fringes; (9) few ulnar tubercles; (10) no knee and heel tubercles, outer tarsal fold bearing one to three indistinct tubercles; (11) two metatarsal tubercles, inner oval, 3x the size of outer conical and elliptical metatarsal tubercle; supernumerary plantar tubercles numerous; (12) all toes with hyperdistal tubercles; toes with narrow lateral fringes; basal toe webbing absent, discs broadly expanded, Toe IV much longer than Toe III (disc on Toe III reaches proximal edge of penultimate subarticular tubercle on Toe IV, disc on Toe V exceeds the distal edge of penultimate subarticular tubercle on Toe IV), discs as expanded as those on fingers (Fig. 4A); (13) SVL 22.8 ± 1.4 mm (20.4–24.8 mm; $n = 15$) in females, 18.5 ± 1.5 mm (15.8–23.9 mm; $n = 39$) in males.

Comparison with other species. Color comparisons are based on digital photos of live specimens, unless otherwise noted. *Pristimantis petersioides* sp. nov. is most similar to other species of the *P. lacrimosus* group, especially *P. petersi* (Lynch & Duellman, 1980), *P. bromeliaceus* (Lynch, 1979), *P. lacrimosus* (Jiménez de la Espada, 1875), *P. schultei* (Duellman, 1990), *P. pastazensis* (Andersson, 1945), and *P. rhodostichus* (Duellman & Pramuk, 1999) (Fig. 5). *Pristimantis petersioides* sp. nov. can only be distinguished from *P. petersi* by differences in advertisement calls. Call duration is shorter in *P. petersioides* sp. nov. 0.25 s (Table 3; 0.19–0.32 s; n = 3) than in *P. petersi*, 0.42 s (0.37–0.46 s; n = 2). Dominant frequencies also differ: 4430.79 Hz (4122–4837.22 Hz; n = 3) in *P. petersioides* sp. nov. and 3956.75 Hz (3836.67–4076.84 Hz; n = 2) in *P. petersi*. Call duration and dominant frequency are static call traits and, therefore, are less variable within species and most reliable to define species boundaries (Köhler et al. 2017). Crucially, the new species and *P. petersi* are not sister species and are separated by large genetic distances: uncorrected pairwise *p*-genetic distances for gene 16S range from 7.9% to 8.4%. *Pristimantis petersioides* sp. nov. can be distinguished from *P. bromeliaceus* by snout shape (rounded to truncate in *P. petersioides* sp. nov. vs. subacuminate in *P. bromeliaceus*), texture of ventral skin (weakly areolate in *P. petersioides* sp. nov. vs. coarsely areolate in *P. bromeliaceus*), iris coloration (reddish coppery in *P. petersioides* sp. nov. vs. brown flecked with gold or bronze in *P. bromeliaceus*), and by having an eyelid with one conical tubercle surrounded by lower tubercles (two to three non-conical tubercles in *P. bromeliaceus*). *Pristimantis petersioides* sp. nov. differs from *P. lacrimosus* (Jiménez de la Espada, 1875) in dorsal coloration (dark greenish brown to pale yellowish green in *P. petersioides* sp. nov. vs. golden brown in *P. lacrimosus*), presence of eyelid tubercles and narrow lateral fringes (both absent in *P. lacrimosus*), and size of outer metatarsal tubercle (3× bigger than the inner metatarsal tubercle in *P. petersioides* sp. nov. vs. 5–6× bigger in *P. lacrimosus*). *Pristimantis petersioides* sp. nov. is also similar to *P. rhodostichus* and *P. schultei* from Peru and Ecuador. It can be distinguished from both by snout shape in dorsal view (rounded to truncate in *P. petersioides* sp. nov. vs. long acuminate in *P. rhodostichus* [Duellman & Pramuk, 1999] and acuminate in *P. schultei*). It can be further distinguished from *P. schultei* by lacking heel tubercles (present in *P. schultei*), and from *P. rhodostichus* by lacking red markings on the dorsum (present in *P. rhodostichus*, Duellman & Pramuk, 1999). Additionally, *P. petersioides* sp. nov. differs from *P. pastazensis* (Andersson, 1945) by snout shape in dorsal view (rounded to truncate in *P. petersioides* sp. nov. *vs.* subacuminate in *P. pastazensis*, Andersson, 1945), tubercles on upper eyelid (one distinct conical tubercle surrounded by lower, indistinct rounded tubercles in *P. petersioides* sp. nov. vs. several minute rounded tubercles in *P. pastazensis*, Andersson 1945), and skin of venter (weakly areolate in *P. petersioides* sp. nov. vs. coarsely granular in *P. pastazensis*, Andersson 1945). For further comparison see Table 4.

Description of the holotype. Adult female (QCAZ58939). Measurements (in mm): SVL 22.02; tibia length 12.07; foot length 10.72; head length 8.82; head width 9.09; eye diameter 2.96; tympanum diameter 1.35; interorbital distance 2.52; upper eyelid width 2.44; internarial distance 1.59; eye-nostril distance 2.59; tympanum-eye distance 0.71. Body slender; head slightly wider than long, wider than body; snout rounded to truncate with rostral papilla in dorsal view, truncate in lateral profile; canthus rostralis distinct, slightly curved in dorsal view; loreal region concave; interorbital space flat, no cranial crests; eye large, protuberant; upper eyelid about 97% of interorbital distance, bearing one subconical tubercle. Tympanic membrane and annulus distinct, rounded, with inconspicuous supratympanic fold, partially obscuring anterodorsal edge; horizontal diameter of tympanum about 13% of head length, separated from eye by a distance about one half tympanum length; choanae large, rounded, not concealed by palatal shelf of maxillary arc; dentigerous processes of vomers prominent, oblique, bearing a transverse row of five teeth; tongue big, elliptical, posterior border slightly notched, 40% of the anterior surface adherent to floor of mouth. Skin on dorsum smooth to shagreen; dorsolateral folds absent; skin on upper flanks bearing scattered low tubercles; skin on belly weakly areolate; skin on throat and chest smooth; discoidal fold ill-defined; skin in upper cloacal region shagreen. Forearms slender bearing low antebrachial tubercle and one subconical ulnar tubercle at the distal half of the forearm; fingers large and slender, all with broadly

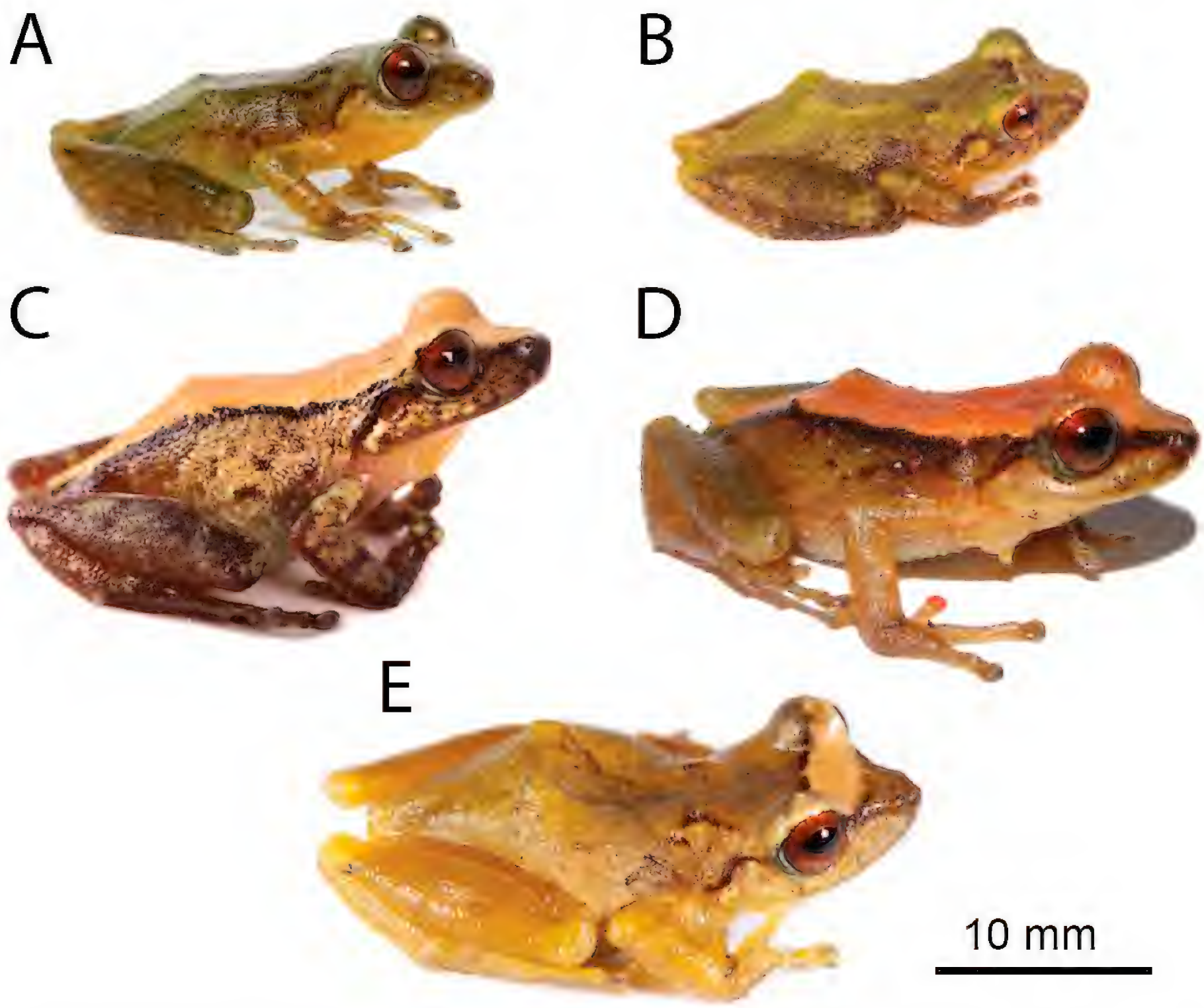

Figure 5. Live specimens of *Pristimantis petersioides* sp. nov. and most similar species. **A** *Pristimantis petersioides* sp. nov., QCAZ58938, adult male (SVL 17.99 mm), **B** *Pristimantis petersi*, QCAZ63455, adult male (SVL 16.49 mm), **C** *Pristimantis* sp., QCAZ62940, adult male (SVL 23.45 mm), **D** *Pristimantis bromeliaceus,* QCAZ56454, adult male (SVL 21.93 mm) **E** *Pristimantis schultei,* QCAZ51551, adult male (SVL 24.60 mm). Photographs by Juan Carlos Sánchez **A**, by David Velalcázar **B**, by Valeria Chasiluisa **C**, by Jorge Brito **D**, by Diego Paucar **E**.

expanded pads, all fingers with discs; fingers bearing narrow lateral fringes; relative lengths of fingers I < II < IV < III; three subarticular tubercles on finger III (Fig. 4B), the most distal we refer as hyperdistal, all the tubercles well defined, round in ventral and lateral view; several supernumerary tubercles present, prominent at the base of the fingers and lower, indistinct at the palmar surface; palmar tubercle bifid, heart-shaped, about the same length and twice the width of elliptical thenar tubercle (Fig. 4B).

Hindlimbs slender; tibia length about 55% of SVL; upper surfaces of hindlimbs smooth; foot length about 48% of SVL, posterior surfaces of thighs smooth, ventral surfaces of thighs slightly areolate; knee and heel lacking tubercles; outer surface of tarsus bearing three low, inconspicuous tubercles, equally distributed along tarsus; toes bearing narrow lateral fringes; webbing between toes absent; discs on toes broadly expanded as those on fingers, rounded; relative lengths of toes: I < II < III < V < IV; Toe V much longer than Toe III (disc on Toe III reaches proximal edge of penultimate subarticular tubercle on Toe IV, disc on Toe V exceeds the distal edge of penultimate subarticular tubercle on Toe IV), subarticular tubercles rounded, simple, elevated; plantar surface with low supernumerary tubercles, bearing four subarticular tubercles (Fig. 4A), inner metatarsal tubercle prominent, elliptical, approximately 3x size of oval and conical outer metatarsal tubercle (Fig. 4A).

Color of holotype in preservative. (Fig. 3C, D) Background color pale grayish cream with scattered, irregular dark brown chevrons, head bearing dark brown supratympanic and canthal stripe, upper lip bearing ill-defined stripe formed by irregular dark brown dots; upper flanks bearing dark brown, irregular flecks and blotches densely distributed; venter, ventral surfaces of forearms and hindlimbs pale creamy white, chest and throat with diminutive dark brown dots uniformly distributed (visible under magnification); ventral surfaces of hands and foot with dense minute dark brown dots, posterior surfaces

Table 3. Quantitative and qualitative characteristics of the advertisement call of *Pristimantis petersioides* sp. nov. from two localities: Sardinayacu, Morona Santiago Province (2.0983°S, 78.1155°W, 1345 m) and Yurugyacu river, Llanganates National Park, Pastaza Province, Ecuador (1.3524°S, 78.0597°W, 1419 m) in comparison with *Pristimantis petersi* from Cocodrilos, Napo Province (0.6710°S, 77.7927°W, 1575 m). Mean is given with range in parentheses. Because the dominant frequency is on the first harmonic, it equals the fundamental frequency.

	***Pristimantis petersioides* sp. nov.**	***Pristimantis petersioides* sp. nov.**	***Pristimantis petersioides* sp. nov.**	***Pristimantis petersi* (not collected)**	***Pristimantis petersi* (not collected)**
	QCAZ58940	**QCAZ59466**	**(not collected)**		
Calls analyzed	47	37	13	4	9
Call duration (s)	0.19 (0.11–0.32)	0.32 (0.16–0.39)	0.25 (0.19–0.34)	0.46 (0.41–0.49)	0.37(0.31–0.44)
Call rate (calls/minute)	11.26 (1.77–18.40)	25.78 (4.52–35.14)	22.64 (12–17.88)	4.24 (1.86–6.62)	9.04 (2.17–33.18)
Call interval (s)	6.21 (3.03–33.66)	2.49 (1.37–13.09)	2.44 (1.82–3.05)	20.16 (8.59–31.73)	11.13(1.41–27.20)
Call rise time (s)	0.03 (0.021–0.036)	0.013 (0.011–0.019)	0.008 (0.004–0.015)	0.064(0.04–0.12)	0.0107(0.07–0.18)
Notes per call	1	1	1	1	1
Fundamental frequency (Hz)	4837.22 (4373.4–5092.6)	4122 (3644.5–4382.8)	4333.1 (4295.9–4392.8)	3846.7(3820.3–3890.6)	4076.8 (3914.1–4242.2)
Dominant frequency (Hz)	1st harmonic	1st harmonic	1st harmonic	1st harmonic	1st harmonic

Table 4. Qualitative morphological characters of species most similar to *P. petersioides* sp. nov.

	Maximun size in females	**Eyelid tubercle**	**Discoidal fold**	**Dorsal snout shape**	**Lateral snout shape**	**TY/ED**	**Knee tubercle**	**Heel tubercle**	**Dorsum**	**Data source**
P. bromeliaceus	27.2 mm	2-3 non conical	prominent	subacuminate	pointed	1/4 to 2/5	small wart	conical	smooth	Lynch , 1979
P. lacrimosus	32.5 mm	absent	evident	rounded	rounded	3/10	absent	absent	finely shagreen	Jiménez de la Espada, 1875
P. petersi	21.1 mm	conical	absent	rounded	truncate	3/5 to 1/2	absent	absent	smooth	Herein
P. petersioides sp. nov.	24.8 mm	conical	ill-defined	rounded to truncate	truncate	2/5 to 1/2	absent	absent	smooth to shagreen	Herein
P. rhodostichus	29.5 mm	several low	prominent	long, acuminate	acutely rounded	2/5	absent	absent	finely shagreen	Duellman and Pramuk, 1999
P. schultei	34.0 mm	several low	not evident	acuminate	inclined posteroventrally	2/5 to 1/2	absent	several low	shagreen	Duellman, 1990

of thighs pale cream to dark brown; iris reddish coppery with fine, dense, black reticulation.

Color of holotype in life. (Fig. 3A, B) Dorsal surfaces yellowish green with scattered, irregular dark brown chevrons; canthal stripe and supratympanic fold black, upper flanks pale cream with dark brown irregular flecks and blotches; venter creamy white; axils pinkish white; ventral surfaces of limbs, thighs yellowish green; iris reddish copper with dark bronze faint horizontal streak and thin irregular black reticulations.

Variation in preservative. (Fig. 6) Adult males (15.79–23.93 mm) are smaller than adult females (20.42–24.81 mm). See Table 5 for measurements of the type series. Males bearing vocal slits and prominent subgular sac, lacking nuptial pads. Skin tuberculation is less noticeable than in live specimens, it can vary from dorsum completely smooth (e.g., QCAZ58943, 59171) to shagreen (e.g., QCAZ59456). Tubercles on flanks remain conspicuous when dorsum is shagreen (e.g., QCAZ59470, 59472) and also upper eyelids tubercles and interorbital tubercle are more evident when dorsum is shagreen or flanks are tuberculated (e.g., QCAZ59456, 59470). Dorsal background coloration in preserved specimens varies from uniform dark brown (e.g., QCAZ58951, 59451, 59461) to pale cream (e.g., QCAZ58881, 58936, 59166). Marks on dorsum varies from scattered, irregular dark brown chevrons that form a triangle extending from the ilium to the scapula (e.g., QCAZ58950, 59468), ill-defined, dark brown flecks and spots (e.g., QCAZ58948, 59472), pale cream middorsal bar from the snout to the cloaca (e.g., QCAZ59456), to black dorsolateral stripes suffused with supratympanic stripes (e.g., QCAZ58943, 59171), with or without dark interorbital bar.

Variation in life. (Fig. 7). Tuberculation pattern varies from dorsum completely smooth (e.g., QCAZ58943, 58951) to dorsum shagreen (e.g., QCAZ58938, 58939), some individuals bear scattered small tubercles on anterior half of dorsum (e.g., QCAZ58880) or have the dorsum densely tuberculated (e.g., QCAZ59463). When dorsum is tuberculated, flanks and limbs usually bear scattered tubercles more conspicuous than those in the dorsum. Similarly, the interorbital tubercle and upper eyelid tubercles are more prominent when the dorsum is tuberculated. There is extensive variation in dorsal coloration (Fig. 7). Dorsum varies from dark greenish brown (e.g., QCAZ59471), bright orange (e.g., QCAZ58943), olive green (e.g., QCAZ58938), to pale yellowish green (e.g., QCAZ58941). Dark marks on dorsum vary from scattered dark brown flecks to irregular brown chevrons that form a triangle that extends from the ilium to the scapula, to ill-defined, dark brown flecks and spots (e.g., QCAZ58948, 59455). Some individuals bear bright orange blotches limited by dark brown contours (e.g., QCAZ58951, 59458), a bright orange middorsal bar that extends from the snout to the cloaca (e.g., QCAZ59456), black dorsolateral stripes suffused with supratympanic stripes (e.g., QCAZ58943). Bright orange to yellow with a darker contour interorbital stripe or bar may be present (e.g., QCAZ59455, 59458, 59462) or absent (e.g., QCAZ58943, 59456). Snout varies from dark green-

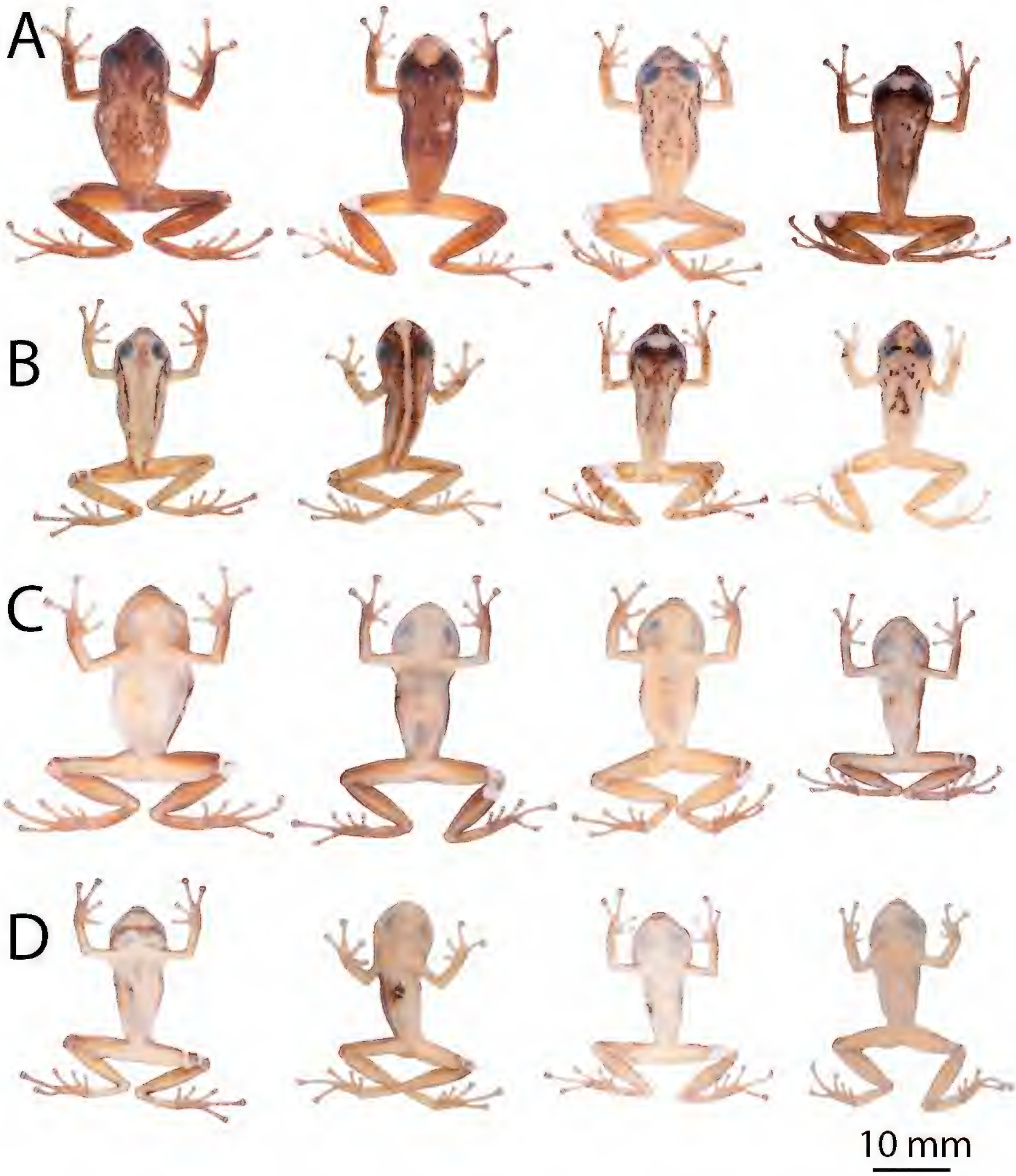

Figure 6. Color variation in preserved individuals of *Pristimantis petersioides* sp. nov. **A** Dorsal view (left to right): QCAZ59461(SVL 22.95 mm), QCAZ59470 (SVL 22.94 mm), QCAZ58939 (SVL 22.02 mm), QCAZ58951 (SVL 19.75 mm), **B** Dorsal view (left to right): QCAZ59171 (SVL 19.50 mm), QCAZ59456 (SVL 19.05 mm), QCAZ59462 (SVL 19.00 mm), QCAZ59468 (SVL 18,35 mm), **C** Ventral view of specimens in (**A**), **D** Ventral view of specimens in (**B**). Photographs by Julio C. Carrión-Olmedo.

ish brown, pale yellowish green to bright orange (e.g., QCAZ59455, 59466, 59471).

Advertisement call. Quantitative measurements of the advertisement call of *Pristimantis petersioides* sp. nov. (QCAZ58940) are shown in Table 3. The call is a metallic click with an average duration of 0.25 s (0.19–0.32 s; n = 3; Fig. 8). The amplitude peak occurs at 20–30 ms and then decreases gradually towards the end (Fig. 8). The calls are repeated at a mean rate of 19.89 calls per minute (11.26–25.78; n = 3). Three or four harmonics are visible, but most of the energy is located on the first one. The dominant frequency (= fundamental frequency) is 4430.79 Hz (4122–4837.22 Hz; n = 3).

Table 5. Morphometric variables of *P. petersioides* sp. nov. and *P. petersi*. Mean ± SD is given with range in parentheses. All measurements are in millimeters.

Variable	*P. petersioides* sp. nov.		*P. petersi*	
	male	female	male	female
	n = 39	n = 15	n = 10	n = 2
Snout-vent length	18.5 ± 1.5 (15.8–23.9)	22.8 ± 1.4 (20.4–24.8)	18.3 ± 1.7 (16.5 –22.7)	20.1 ± 1.4 (19.1–21.1)
Tibia length	9.8 ± 0.8 (8.3–11.9)	11.7 ± 1.0 (8.5–12.6)	9.6 ± 0.6 (8.6–11.0)	10.7 ± 1.6 (9.6–11.8)
Foot length	8.6 ± 0.9 (6,9–10.7)	10.6 ± 0.7 (8.8–11.6)	8.6 ± 0.7 (7.8–10.1)	10.0 ± 0.3 (9.8–10.2)
Head length	6.5 ± 0.6 (5.3–7.8)	8.0 ± 0.6 (7.2–9.1)	6.1 ± 0.5 (5.5–7.1)	7.1 ± 0.5 (6.8–7.5)
Head width	7.0 ± 0.6 (5.9–8.5)	8.8 ± 0.5 (8.0–9.5)	6.9 ± 0.5 (6.3–8.1)	7.7 ± 0.4 (7.5–8.0)
Eye diameter	2.6 ± 0.2 (2.2–3.1)	3.0 ± 0.3 (2.4–3.5)	2.6 ± 0.2 (2.4–3.1)	2.8
Tympanum diameter	0.9 ± 0.1 (0.7–1.1)	1.1 ± 0.2 (0.8–1.4)	0.9 ± 0.1 (0.7–1.0)	1.1 ± 0.1 (1.0–1.2)
Interorbital distance	2.2 ± 0.2 (1.9–2.6)	2.6 ± 0.2 (2.4–3.0)	2.2 ± 0.1 (2.0–2.5)	2.6 ± 0.2 (2.4–2.7)
Upper eyelid width	2.2 ± 0.3 (1.6–2.6)	2.6 ± 0.2 (2.3–3.0)	2.1 ± 0.4 (1.7–2.9)	2.27
Internarial distance	1.4 ± 0.1 (1.2–1.7)	1.7 ± 0.1 (1.5–2.0)	1.5 ± 0.2 (1.3–1.7)	1.6 ± 0.2 (1.4–1.7)
eye–nostril distance	1.9 ± 0.2 (1.6–2.5)	2.5 ± 0.2 (2.2–2.8)	2.1 ± 0.3 (1.8–2.9)	2.3 ± 0.4 (2.0–2.6)

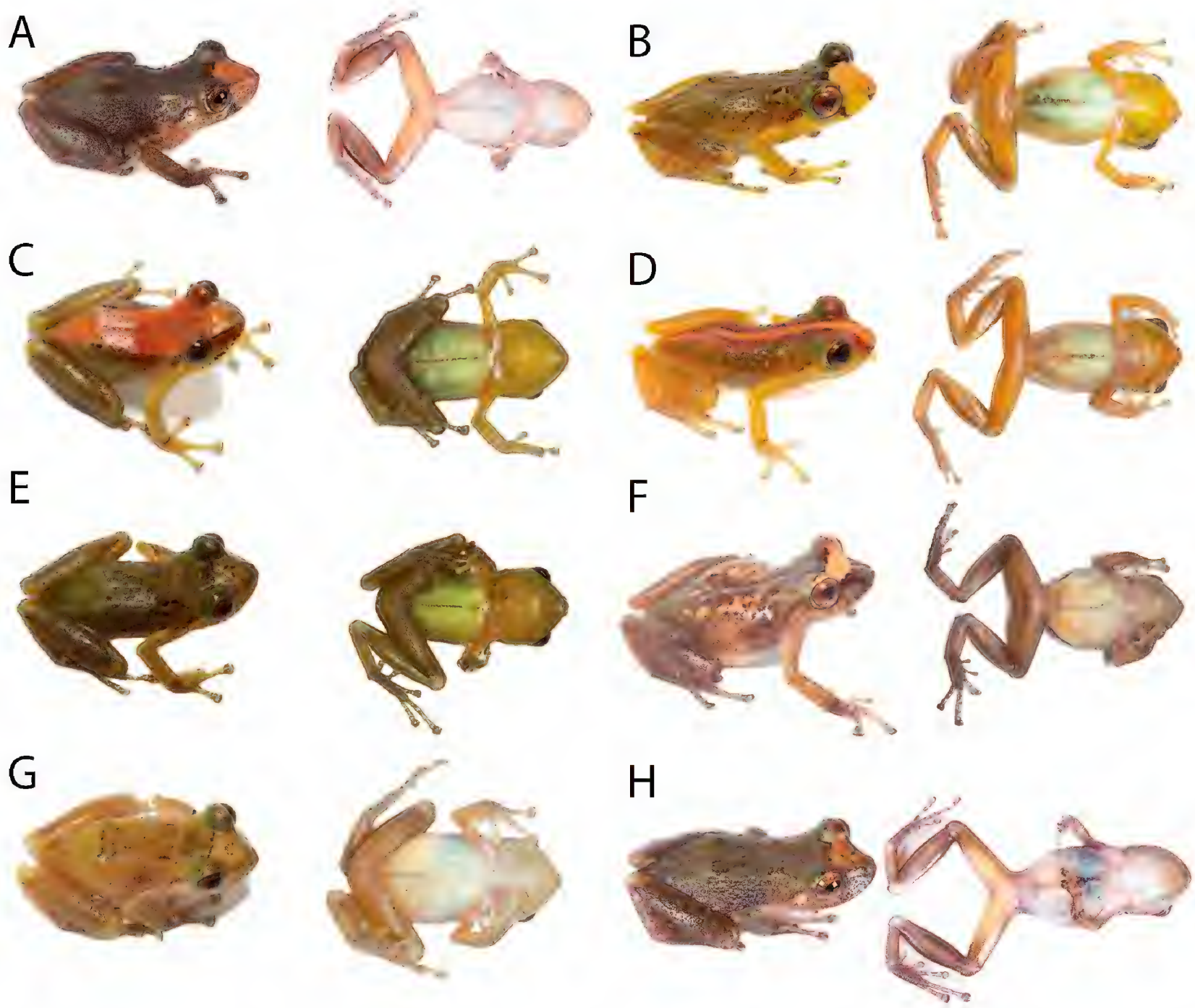

Figure 7. Variation in live adult individuals of *Pristimantis petersioides* sp. nov. **A** QCAZ59471 (SVL 17.45 mm), **B** QCAZ59455 (SVL 18.2 mm), **C** QCAZ58943 (SVL 17.73 mm), **D** QCAZ59456 (SVL 19.05 mm), **E** QCAZ58938 (SVL 17.99 mm), **F** QCAZ59458 (SVL 21.84 mm), **G** QCAZ58941 (SVL 20.42 mm), **H** QCAZ59466 (SVL 19.06 mm). Dorsolateral view on the left, ventral view on the right. Photographs **A** and **H** by Santiago R. Ron, **B–G** by Juan Carlos Sánchez.

Distribution and natural history. *Pristimantis petersioides* sp. nov. is known from six localities in the eastern Andean slopes of central Ecuador between 1221–2300 m (Fig. 9). It inhabits the Eastern Andean Foothills Forest and Eastern Montane Forest natural regions (as defined by Ron et al. 2019). It has been recorded in primary forest and, less frequently, in secondary forest. Individuals were found during nocturnal surveys, usually perching on ferns, herbs, or *Heliconia* leaves, branches, or inside bromeliads up to 350 cm above the ground, usually near water bodies. Three amplectant pairs were found on January and February 2015 in Sardinayacu and Zarentza.

Etymology. The specific epithet is a masculine noun in apposition. The suffix *oides* is derived from the Greek *eidos* meaning similar. The name makes reference to the

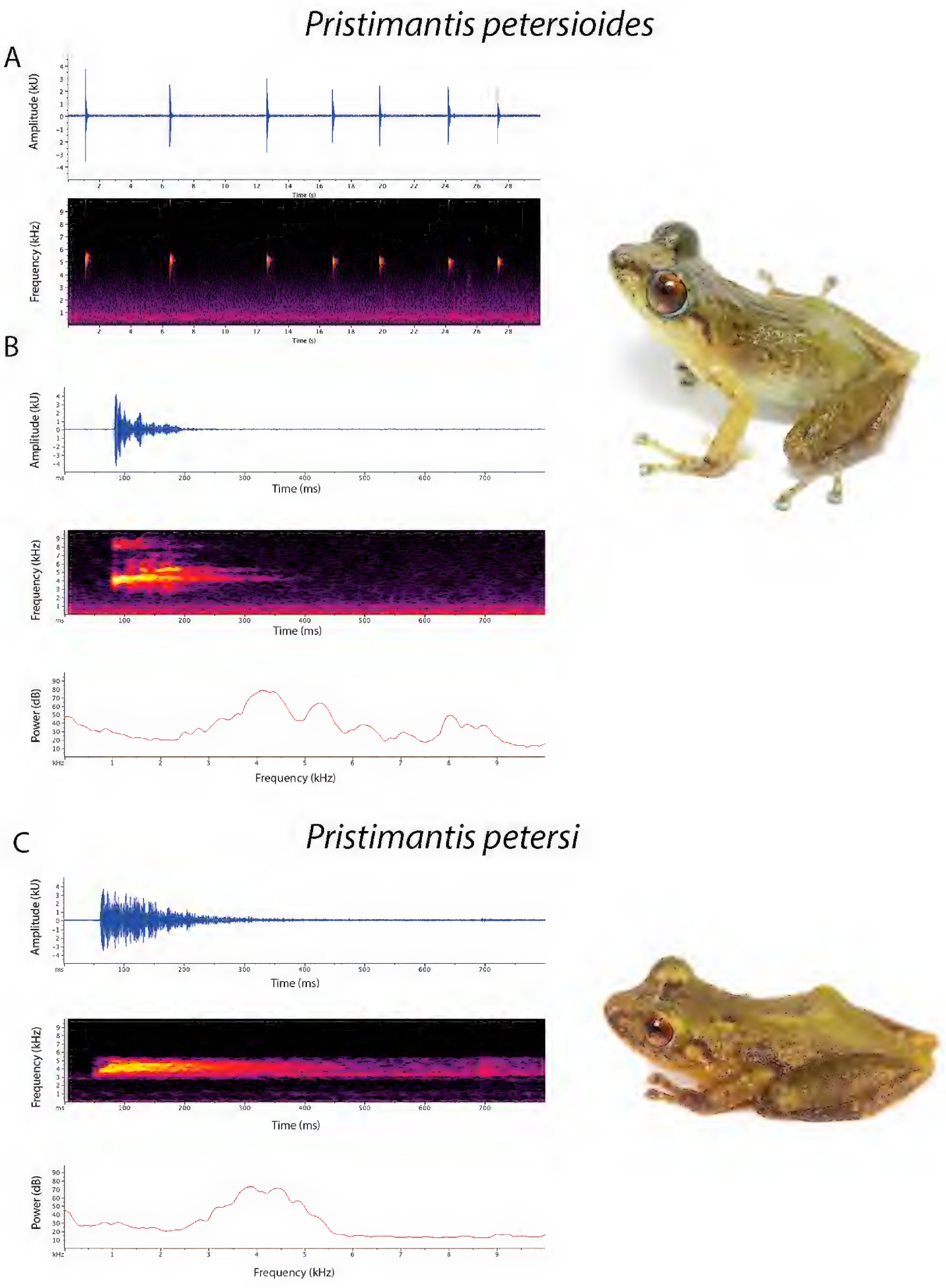

Figure 8. Advertisement calls of *Pristimantis petersioides* sp. nov. and *Pristimantis petersi*. (**A**) oscillogram and spectrogram of a call series of *Pristimantis petersioides* sp. nov., (**B**) oscillogram (top), spectrogram (middle), and power spectrum (bottom) of a single call of *Pristimantis petersioides* sp. nov. and (**C**) oscillogram (top), spectrogram (middle), and power spectrum (bottom) of a single call of *Pristimantis petersi*.

similarity between the new species and its sister species, *Pristimantis petersi*.

Conservation status. Four out of six known localities are inside National Parks (Sardinayacu in Parque Nacional Sangay and Ankaku, Zarentza and Salcedo-Tena road in Parque Nacional Llanganates); nonetheless, based on a vegetation cover map (Ministerio del Ambiente 2018a) and a deforestation map 2016–2018 (Ministerio del Ambiente 2018b), Zarentza is < 1 km from deforested areas for agriculture. At the year of collection (2009) the locality at Salcedo-Tena highway was in a forested region with small, deforested patches at distances > 2.5 km (based on a 2008 deforestation map by Ministerio de Ambiente). Sardinayacu, refuge 3 occur > 6 km from pastures, while Sardinayacu, refuge 1 is < 0.5 km from deforested areas for agriculture.

In Sardinayacu, this species was one of the most common during surveys (24 individuals found in 9 days by 13 people) which suggest it can be locally abundant. Brito et al. (2017) also reported abundant populations in the upper basin of the Upano river, Sangay National Park, Morona Santiago Province (referred both as "*Pristimantis petersi*" and also "*P.* aff. *petersi*"). Its extent of occurrence is

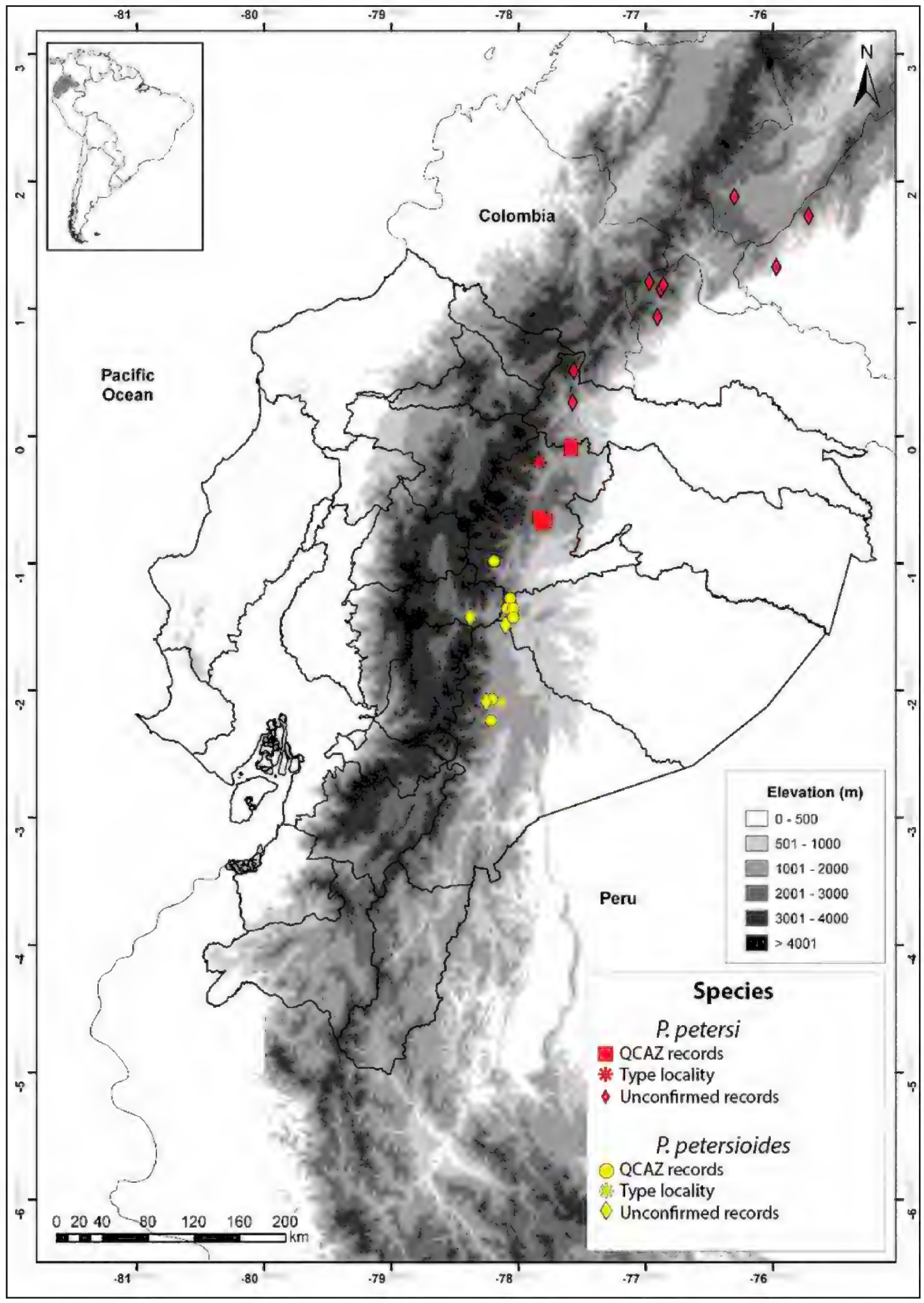

Figure 9. Records of *Pristimantis petersioides* sp. nov. (yellow) and *P. petersi* (red). Confirmed records are based on specimens deposited at the Museum of Zoology, Pontificia Universidad Católica del Ecuador. Unconfirmed records (diamonds) from Brito et al. (2017), Lynch and Duellman (1980), Mueses-Cisneros (2005), and Stuart et al. (2008).

1402 km^2 (based on a minimum convex polygon). Despite being locally abundant, we consider *Pristimantis petersioides* sp. nov. to be in the Red List category Vulnerable (VU) following B1, B2ab(iii) IUCN criteria because: (i) it is only known from six localities (sensu IUCN 2017), (ii) its Extent of Ocurrence is less than 5000 km^2 (1433 km^2); and approximately 9% of its Extent of Ocurrence has been affected by deforestation, human settlements and agriculture (Fig. 10).

Remarks. *Pristimantis petersioides* sp. nov. differs from *P.* sp. (QCAZ 60398, from Bombuscaro) by the snout shape (in dorsal view, rounded in *P. petersioides* sp. nov., subacuminate in *P.* sp. QCAZ60398), venter texture (weakly areolate in *P. petersioides* sp. nov.; coarsely areolate in *P.* sp. QCAZ60398), presence of small rostral papilla (absent in *P.* sp. QCAZ60398); furthermore, *P. petersioides* sp. nov. bears a complete, rounded tympanic annulus, weakly obscured posterodorsally by a thin supratympanic fold (tympanic annulus concealed posterodorsally by a thick supratympanic fold in *P.* sp. QCAZ60398). It differs from *P. nankints* by snout shape in dorsal view (rounded to truncate in *P. petersioides* sp. nov. vs. acuminate in *P. nankints)*.

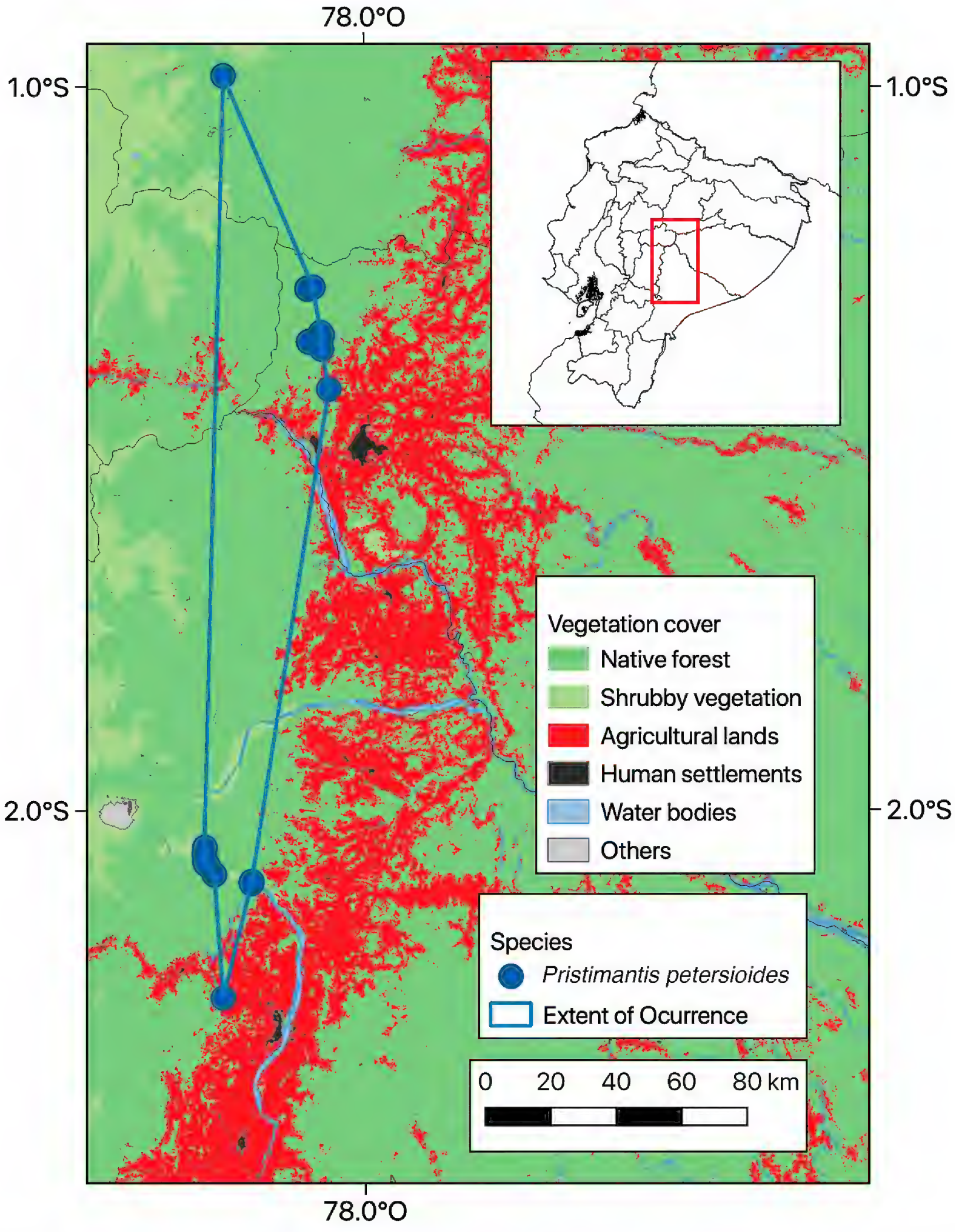

Figure 10. Records of *Pristimantis petersioides* sp. nov. (blue circles) and its Extent of Ocurrence. Colors indicate vegetation cover based on Ministerio de Ambiente (2018a).

Discussion

On the identity of *Pristimantis petersi*

Pristimantis petersi was considered to have a wide distribution from the central Andes of Colombia in Caquetá, Huila, and Putumayo (Lynch and Duellman 1980; Mueses-Cisneros 2005; Stuart et al. 2008), to the eastern slopes of the Ecuadorian Andes, from Sucumbíos to Morona Santiago Provinces (Brito et al. 2017; Ron et al. 2019). Herein, we show that it was composed of two species which appear to be allopatric, south and north of the Quilindaña paramos in Napo Province.

Lynch and Duellman (1980) remark of size differences between populations from the north and south of "*P. petersi*" was not supported in our data but their suspicion of the distinctiveness of the populations from the Pastaza trench was correct. Based in our review, we tentatively consider *Pristimantis petersi* as distributed from the central Andes of Colombia to Napo Province (Fig. 9). We recommend verifying the identity of Colombian populations using genetic data. Recent reviews of Andean *Pristimantis* indicate that species usually have a restricted distribution (e.g., Páez and Ron 2019). The geographic distance of Colombian populations (up to 320 km from the type locality) suggest that, at least some of them, could represent a separate species.

Guayasamin and Funk (2009) reported an abundant population of "*Pristimantis* cf. *petersi*" at Yanayacu Biological Station. Examination of voucher specimens deposited at the QCAZcollection indicate that they are not conspecific with *P. petersi* nor *P. petersioides* sp. nov.

Our results and those of previous systematic reviews (show that eastern montane forests still harbor many undescribed species of *Pristimantis*. Similar findings have been previously reported by Ortega et al. (2015), Páez and Ron (2019), and Ron et al. (2020). As in previous reviews (e.g., Restrepo et al. 2017; Páez and Ron 2019), we also found broad intraspecific and intrapopulation variation in dorsal color within *P. petersioides* sp. nov. (Figs 6, 7) and *P. petersi* (Fig. 11). This large intraspecific and intrapopulation variation hinders the use of dorsal coloration for diagnosis between both species. Most individuals have greenish dorsal color which is characteristic of several species of the *P. lacrimosus* group. We did not find diagnostic morphological characters to distinguish the new species from *Pristimantis petersi,* which highlights the importance of including molecular and bioacoustic data to clarify species identity.

Use of bioacoustics for species delimitation

Similar to Páez and Ron (2019), our morphometric analysis was of little help to distinguish closely related species of *Pristimantis*. In contrast, advertisement calls and genetic data indicate that *P. petersioides* sp. nov. represents a lineage independent from *P. petersi*. We found differences with little or no overlap in two static call traits, call duration and dominant frequency (Köhler et al. 2017). Moreover, differences in call frequency are likely an underestimate because the calling males of *P. petersioides* sp. nov. were larger than those from the recorded chorus of *P. petersi*. Because there is an inverse relationship between body size and call frequency (Gerhardt and Huber 2002), the higher frequency of the call of *P. petersioides* sp. nov. would be likely higher after a size correction.

Bioacoustic comparisons are of importance for taxonomy because advertisement calls mediate species recognition and mate choice (e.g., Ryan and Rand 1995). It has been widely accepted that calls are among the most useful characters differentiating closely related anuran species (Vences and Wake 2007). However, calls have been of limited use in the taxonomy of *Pristimantis* (Duellman and Lehr 2009). Our study and some recent works (Hutter and Guayasamin 2015; Páez and Ron 2019; Székely et al. 2020) highlight the usefulness of bioacoustic characters in *Pristimantis* taxonomy. Future taxonomic reviews will benefit from a more comprehensive knowledge of advertisement calls in *Pristimantis*.

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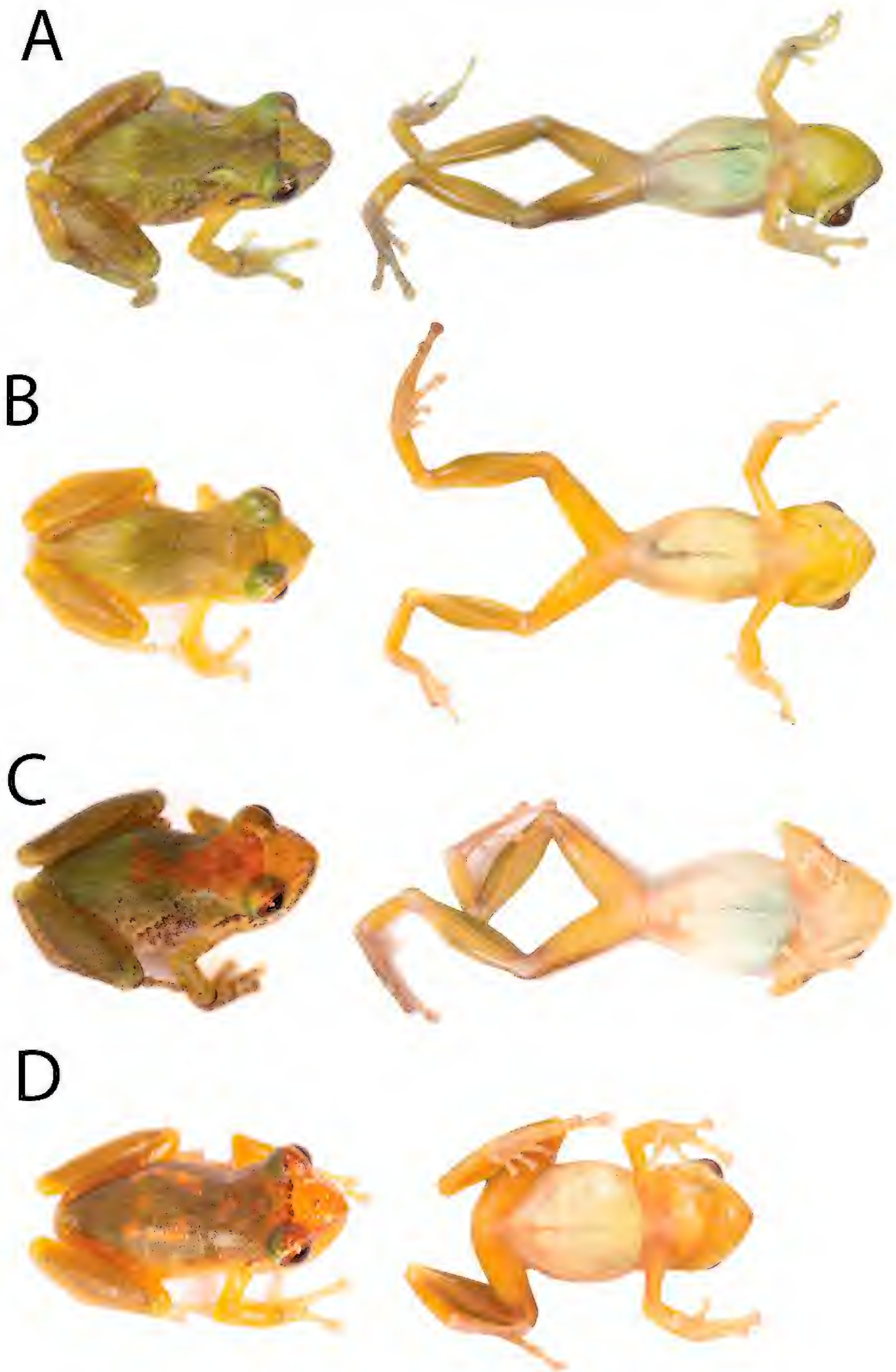

Figure 11. Variation in live adult individuals of *Pristimantis petersi*. **A** QCAZ63452 (SVL 17.42 mm), **B** QCAZ63453 (SVL 17.75 mm), **C** QCAZ63454 (SVL 21.11 mm), **D** QCAZ63456 (SVL 19.11 mm). Photographs by Santiago R. Ron **A**, by David Velalcázar **B–D**.

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Supplementary material 1

Collection data for Museum specimens examined in the morphological comparisons

Authors: Julio C. Carrión-Olmedo, Santiago R. Ron
Data type: Collection data
Explanation note: All specimens are deposited at the amphibian collection of the Zoological Museum (QCAZ), Pontificia Universidad Católica del Ecuador in Quito, Ecuador.
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Link: https://doi.org/10.3897/evolsyst.5.62661.suppl1

Supplementary material 2

Best-fit models of DNA evolution for partitions of the phylogenetic analyses

Authors: Julio C. Carrión-Olmedo, Santiago R. Ron
Data type: Phylogenetic
Explanation note: Models were chosen according to the BIC criterion.
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Link: https://doi.org/10.3897/evolsyst.5.62661.suppl2

Supplementary material 3

Mitochondrial DNA and RAG1 phylogenetic trees

Authors: Julio C. Carrión-Olmedo, Santiago R. Ron
Data type: Phylogenetic
Explanation note: The *Pristimantis lacrimosus* species group is shown in green.
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